

Sense and specificity in neuronal calcium signalling

Robert D Burgoyne and Lee P. Haynes

Department of Cellular and Molecular Physiology, The Physiological Laboratory, Institute of Translational Medicine, University of Liverpool, Crown Street, Liverpool, L69 3BX, United Kingdom.

Corresponding author: Robert D. Burgoyne

Email: burgoyne@liv.ac.uk

Tel: +44-151-794-5305

ABSTRACT

Changes in the intracellular free calcium concentration ($[Ca^{2+}]_i$) in neurons regulates many and varied aspects of neuronal function over time scales from microseconds to days. The mystery is how a single signalling ion can lead to such diverse and specific changes in cell function. This is partly due to aspects of the Ca^{2+} signal itself including its magnitude, duration, localisation and its persistent or oscillatory nature. The transduction of the Ca^{2+} signal requires Ca^{2+} binding to various Ca^{2+} sensor proteins. The different properties of these sensors are important for differential signal processing and determine the physiological specificity of Ca^{2+} signalling pathways. A major factor underlying the specific roles of particular Ca^{2+} sensor proteins is the nature of their interaction with target proteins and how this mediates unique patterns of regulation. We review here recent progress from structural analyses and from functional analyses in model organisms that have begun to reveal the rules that underlie Ca^{2+} sensor protein specificity for target interaction. We discuss three case studies exemplifying different aspects of Ca^{2+} sensor/target interaction.

Key words:

Caenorhabditis elegans

Ca²⁺-binding proteins

Ca²⁺ channel

Ca²⁺ sensors

NCS-1

Neuronal signalling

Abbreviations:

CaBP, calcium-binding protein; DREAM, downstream regulatory element antagonist modulator; CDF, Ca²⁺-dependent facilitation; CDI, Ca²⁺-dependent inhibition; GCAP, guanylyl cyclase activating protein; IL1RAPL1, interleukin1 receptor accessory protein-like 1 protein 1; KChIP, potassium channel interacting protein; NCS, neuronal calcium sensor; NCX, sodium/calcium exchanger; VGCC, voltage-gated Ca²⁺ channel, VILIP, visinin-like protein

1. Introduction

The intracellular free calcium concentration ($[Ca^{2+}]_i$) is tightly regulated through multiple mechanisms in neurons [1] and changes in $[Ca^{2+}]_i$ have crucial roles in the control of normal neuronal function [2]. In addition, abnormalities in Ca^{2+} signalling have been implicated in many aspects of neuropathology, neurodegeneration [3-5] and in psychiatric disorders [6, 7]. The mechanisms that elevate $[Ca^{2+}]_i$ include entry of extracellular Ca^{2+} through voltage-gated Ca^{2+} channels and release from intracellular stores such as the endoplasmic reticulum (ER), lysosomes and mitochondria. $[Ca^{2+}]_i$ can be reduced by sequestration into the same cellular organelles and by extrusion across the plasma membrane and these mechanisms have been well characterised [1, 8]. Large numbers of studies on the localisation, magnitude and time course of $[Ca^{2+}]_i$ fluxes in neurons have been published. Changes in $[Ca^{2+}]_i$ can be local or global, highly transient, oscillatory or persistent. Localisation of $[Ca^{2+}]_i$ changes to particular neuronal compartments is important for the generation of specific neuronal responses. For example, certain stimuli strictly require nuclear rather than cytoplasmic changes in $[Ca^{2+}]_i$ [9] and also highly localised $[Ca^{2+}]_i$ changes restricted to dendritic spines may play an important role in activity-dependent synaptic plasticity [10, 11]. The physiological effects of elevation of $[Ca^{2+}]_i$ can be manifest in microseconds (neurotransmitter release), milliseconds (channel facilitation or inactivation), or over much longer time scales leading to changes in gene expression [12, 13] and effects on synaptic plasticity [14], neuronal development [15] learning [16], neuronal survival and cell death.

Clearly Ca^{2+} can influence many aspects of neuronal function with the same fundamental signalling ion being used to produce a variety of subtle and distinct changes. It is known, for example, that Ca^{2+} entry through different types of plasma membrane channels can affect changes in gene expression by different modes of signalling [17, 18]. Of note here is the fact that even different classes of voltage-gated Ca^{2+} channels (VGCCs) in the same neurons are coupled through distinct signalling pathways to changes in gene expression [19]. Ca^{2+} signals are translated into changes in cellular function through various types of Ca^{2+} sensor proteins that in general terms detect increases in $[Ca^{2+}]_i$ by becoming loaded with Ca^{2+} , undergo a conformational change and then interact with and regulate various target proteins. The subtlety of neuronal Ca^{2+} signalling is underpinned by differential signal processing by these Ca^{2+} sensor proteins which in turn determines the physiological specificity of Ca^{2+} signalling pathways.

Neurons express a large number of Ca^{2+} sensor proteins [20] ranging from synaptotagmin I which is the essential Ca^{2+} -sensor for fast (microsecond) neurotransmitter release [21], through the annexins [22] to many different EF-hand containing proteins [20]. The EF-hand

is a highly conserved Ca^{2+} -binding motif [23] which is present, for example, in the ubiquitous Ca^{2+} sensor protein calmodulin [24]. Calmodulin has numerous targets for regulation and is known to have multiple functions in neurons acting via various targets including Ca^{2+} /calmodulin-dependent protein kinase II [25]. Other EF-hand containing Ca^{2+} sensors expressed in neurons include the calcium-binding protein (CaBP) family [26, 27] and the neuronal calcium sensor (NCS) proteins [28-30].

The differential processing of neuronal Ca^{2+} -signals is impacted upon by multiple aspects of the properties of Ca^{2+} sensors and we suggest that they have a key role in determining signalling specificity. Factors that influence differential Ca^{2+} signalling include varied expression levels of sensors between neuronal cell types [31], differences in subcellular localisation, affinity and dynamics of Ca^{2+} -binding [32], association with protein signalling complexes [33, 34], regulation and variations in stoichiometry of binding with target proteins [35, 36] and specificity of target protein interaction. Appreciation of the contribution of Ca^{2+} -sensors to signalling specificity requires analysis of both the Ca^{2+} -sensors and their target interactions at a structural level [24, 37]. The rules underlying the sensing and specificity of Ca^{2+} signalling in neurons have begun to emerge in recent years but still remain to be fully understood. In this review we will present three case studies to illustrate current understanding of the molecular and structural basis of the contribution of the properties of Ca^{2+} sensors to differential neuronal signal processing.

2. Case Study 1 – NCS-1, a Ca^{2+} sensor with multiple specific targets.

NCS-1 is a member of the neuronal calcium sensor family that is encoded by 14 genes in mammals. Of these, recoverin and the guanylyl cyclase activating proteins (GCAPs) 1-3 have specialised roles in Ca^{2+} regulation of phototransduction. One other subfamily, consisting of the visinin-like proteins (VILIPs) 1-3, neurocalcin δ and hippocalcin has less well defined functions. Hippocalcin, as suggested by its name, has a very restricted pattern of expression mainly in hippocampal neurons where it shows dynamic membrane association (the Ca^{2+} myristoyl switch [38]) in response to Ca^{2+} -elevation [39] and neuronal activity [40, 41] including activity-dependent translocation into dendritic spines. Hippocalcin has been implicated as a Ca^{2+} sensor in long-term depression [42, 43] and the gating of channels underlying a slow after-hyperpolarization current [44]. VILIPs also show the Ca^{2+} myristoyl switch [45] and may have multiple roles including regulation of receptor trafficking [46]. The potassium-channel interacting proteins (KChIPs) have all been implicated in the gating [47] and trafficking [48] of A-type potassium channels but show differential expression in different classes of neurons [49, 50]. One of them, KChIP3 also known as

calsenilin/DREAM regulates presenilin function [51] and can act as a transcriptional repressor [52]. The other KChIPs may share the DREAM activity [53] but KChIP3 is specific amongst the KChIPs in increasing regulated secretion and down regulating the $\text{Na}^+/\text{Ca}^{2+}$ exchanger NCX3 [54].

NCS-1 appears to be expressed in most neuronal cell types [55] [31] and studies in various organisms have determined that it has multiple physiological functions [29, 56-58]. Some of the roles of NCS-1 may be specific to certain organisms such as its particular role in temperature-dependent behaviours in *Caenorhabditis elegans* as a consequence of its restricted neuronal expression [59-61]. One of two encoded NCS-1 proteins generated through gene duplication (*ncs-1a*) is required for semi-circular canal formation in the zebrafish inner ear [62] and NCS-1 (frequenin, Frq) is required for the development of synaptic boutons in *Drosophila* [56] the organism in which NCS-1 was first discovered [63]. In addition, in *Saccharomyces cerevisiae* NCS-1 (Frq1) is essential for survival as a consequence of its requirement for activation of the phosphatidylinositol-4-kinase Pik1 [64] despite its absence not being lethal in other organisms. In mammalian cells NCS-1 regulates Ca^{2+} -dependent exocytosis [65], long-term depression [66] axonal growth and neuronal regeneration [67] and channel function [68]. In mice, knock-out of NCS-1 has relatively subtle effects but results in an increase in anxiety and depressive behaviour [69]. Selective overexpression of NCS-1 in adult mouse dentate gyrus neurons promoted a form of exploratory behaviour and enhanced acquisition of spatial memory [70].

NCS-1 has many known interacting partners [71] (Fig 1) some of which are unique for NCS-1 but some that are also regulated by other Ca^{2+} sensors particularly calmodulin [72]. Some of the interactions are known only from *in vitro* binding assays and so their biological importance is unclear. It is possible that those binding partners that are also calmodulin targets *in vitro* [72] are not regulated by NCS-1 under physiological conditions [73]. There are, however, several physiological effects of NCS-1 that can be attributed directly to one of its identified target proteins (Table 1). Two documented NCS-1 interactions are of possible clinical significance. The potential importance of the regulation of dopamine D2 receptors by NCS-1 whereby NCS-1 inhibits D2 receptor internalisation (Fig. 2) [74] stems from the fact that dopamine is of key importance for signalling within the CNS and in addictive behaviour [75, 76]. Regulation of D2 receptors by NCS-1 has been shown to underlie the effect of overexpression of NCS-1 on spatial memory acquisition [70]. Importantly, dopamine D2 receptors are the targets for all known effective antipsychotic drugs [77]. Interestingly NCS-1 is up-regulated in patients with bipolar disorder or schizophrenia [78] and in response to antipsychotic drugs [79] and is genetically associated with cocaine addiction [80] believed to be linked to effects of cocaine on dopamine transporters [81]. Recently NCS-1 has been shown

to be required for an adaptive response to dopaminergic agonists in substantia nigra neurons and, coupled with its up-regulation in the substantia nigra from Parkinson's disease patients, this has resulted in the suggestion that it could be a target for modifying the vulnerability of neurons in the substantia nigra to neurodegeneration [82]. The binding of NCS-1 to the D2 receptor involves the very short cytoplasmic C-terminal domain of the receptor [74]. This interaction has been partially characterised using structural approaches [83] and this may allow exploration of the interaction as a therapeutic drug target.

The other clinically important interaction is with the interleukin1 receptor accessory protein-like 1 protein (IL1RAPL1) which appears to be specific for NCS-1 [84]. Mutations in IL1RAPL1 have been shown to result in X-linked mental retardation [85, 86] and also have been linked to autistic spectrum disorder (ASD, [87]). Interestingly the latter study also identified a mutation (R120Q) within NCS-1 in an individual with ASD. This mutation was found to cause a functional deficit in NCS-1 [88] that appeared to be related to a change in the structural dynamics of the C-terminus of the protein [88]. However, the physiological relevance of this mutation and its exact relationship to the disease phenotype remains to be established.

One key question is what determines the specificity of target recognition for NCS-1 over other Ca^{2+} sensors and more particularly other members of the NCS protein family which have their own specific targets? The NCS proteins show differences in membrane-association with some such as recoverin and hippocalcin showing transient membrane-association through their Ca^{2+} /myristoyl switch mechanism [38, 39, 45] whereas others including KChIP3 and KChIP4 are predominantly cytoplasmic [54]. Others including KCIP1 and mammalian NCS-1 appear to be constitutively membrane targeted due to their N-terminal myristoylation [89, 90] but note however, that Ncs-1 from fission yeast has been shown to have the Ca^{2+} /myristoyl switch [91]. The NCS proteins can, however, be very closely related to each other at a protein sequence level (> 60% identity). Structural analyses of several NCS proteins have shown common features with highly similar main chain topologies [37]. The gross tertiary topology of NCS proteins fails therefore to adequately explain target specificity and so analyses of complexes of NCS proteins with peptides derived from their target proteins have been undertaken in an effort to better address this question. These studies have highlighted both common and distinct aspects of target recognition that help explain both NCS protein promiscuity and specificity. A common feature is that on Ca^{2+} binding the NCS proteins undergo a conformational change that exposes a hydrophobic groove within which one or two helices from target proteins can bind. Once Ca^{2+} loaded the NCS proteins undergo relatively limited structural changes on target

ligand binding unlike the more dramatic changes seen in calmodulin conformation on ligand binding (described below).

Key hydrophobic amino acids within the hydrophobic groove of the NCS proteins that are conserved across the whole NCS family are directly involved in the target interactions in multiple NCS proteins (Fig 3). Despite this similarity in the mechanism for target protein binding there are structural differences that will determine specificity [37] [30]. There are differences in the overall size of the exposed hydrophobic groove (which may require some movement of the C-terminal tail of the protein) so that two or only one helix can bind. Also, differences in the distribution of surrounding surface exposed charged residues influences binding of specific ligands. In addition, there are key differences in the role of the C-terminal tail. In the case of KChIP1 [92, 93] and recoverin [94] the C-terminal proximal pocket of the groove is partially occluded by the C-terminal tail of the NCS protein allowing only a single helix to bind. In recoverin, amino acids in the C-terminal tail are actually required to make direct contact with the rhodopsin kinase target peptide for high-affinity binding [95]. In one of the published structures of NCS-1 the C-terminal tail appears to be able to sit in the hydrophobic groove in the absence of ligand [96]. This is likely an auto-regulatory mechanism to control target binding as the groove is fully exposed in another published structure of human NCS-1 [97]. In addition, in the structure of the yeast protein in complex with Pik1, two helices of Pik1 were bound within a fully exposed hydrophobic groove and the C-terminus appeared disordered suggesting that the C-terminal tail can undergo a conformational change to allow its movement out of the groove on ligand binding [91, 98].

The structural studies on NCS protein/ target interaction have been based on the use of short peptides derived from the target protein. These studies have provided a consistent picture in which conserved hydrophobic residues are crucial. Nevertheless, it is important to assess the significance of the structural data using a physiological model. This would, for example, allow a test of requirement for the key residues in the hydrophobic groove and also the requirement for the C-terminal tail for NCS-1 function. This was approached using an assay for temperature dependent locomotion in *C. elegans* [99] that requires NCS-1 function in the pair of AIY neurons in the worm. Using an NCS-1 null mutant worm strain an assay was developed for functional reconstitution. Key amino acids were then mutated in the N- and C-terminal parts of the hydrophobic groove (Fig 3) and the mutant proteins expressed in the null background. The findings from this assay were fully consistent with the structural data and showed that for normal NCS-1 function in this behavioural assay hydrophobic residues in both the N- and C-terminal parts of the hydrophobic groove were required [60]. In addition, full functional reconstitution occurred after truncation of the C-terminus of NCS-1 and indeed truncation of residues 169-191 to remove the entire C-terminal helix resulted in a

gain of function [60]. These findings are consistent with the notion that the C-terminal tail of NCS-1 unlike that in for recoverin is not essential for direct target interactions but instead may have an auto-inhibitory role by binding with the C-terminal part of the hydrophobic groove in the absence of a suitable binding ligand [96] and this may prevent binding of inappropriate ligands.

Evidence for a role in specificity determination of residues surrounding the hydrophobic groove has come from analysis of *Drosophila* frequenins. In *Drosophila* species a gene duplication event generated two genes encoding closely similar proteins known as Frq1 (the originally identified frequenin [63]) and Frq2 [100]. Both isoforms affect synaptic transmission and synaptic bouton number [101]. Frq1 and Frq2 differ by only 10 amino acids and it was unclear whether they would have distinct functions. Recent work has now identified the guanine nucleotide exchange factor Ric8a as a binding partner for Frq2 but not Frq1 and functional analyses suggest that the interaction is involved in the physiological roles of Frq2 in the fly [102]. The interaction with ric8 was conserved in the human proteins [102]. From examination of the crystal structure of Frq2 a small number of solvent exposed amino acids that differed between the two frequenins were highlighted as being potentially important for target recognition and mutagenesis of Frq1 established that the amino acids R94 and T138 located at the edge of the hydrophobic groove were required for Ric8a binding. Incidentally, removal of the C-terminal helix of Frq2 increased its binding to Ric8a consistent with the findings on the functional effect of deletion of the C-terminal tail of NCS-1 in *C. elegans* [60]. These findings provide clear evidence in support of the structural models for NCS protein/target specificity [37].

3. Case Study 2 – CaBPs, Ca²⁺ sensors with targets in common with and distinct from calmodulin

The CaBP proteins in humans are encoded by 6 functional genes (Fig 4) with CaBP1 and CaBP2 existing as variants derived from alternative splicing [26, 27, 103, 104]. They have a higher level of similarity to calmodulin than do the NCS proteins but have divergent N-terminal domains that differ between the CaBPs and which do not occur in calmodulin and also have a specific larger linker region between the N- and C-terminal EF-hand domains. Certain of the CaBPs can be membrane-associated through N-terminal myristoylation or possession of a C-terminal transmembrane domain (Fig 4). Both CaBP1/caldendrin and calmodulin can interact with VGCCs. In addition, CaBP1 has been shown to bind and regulate InsP₃ receptors [105] which also bind calmodulin [106]. Originally it was suggested

that CaBP1 activates the receptor but subsequent work has established that instead it has an inhibitory effect on receptor function in response to InsP_3 [107, 108].

CaBP1 is similar to calmodulin in that it has distinct N- and C-terminal lobes [109-111]. Each contains two EF hands but unlike calmodulin the second EF hand is non-functional in CaBP1. The structure of CaBP1 was derived from analyses of the crystal structure of a form with Ca^{2+} -bound to EF3 and EF4 [109-111] and an NMR solution structure of a form with Ca^{2+} bound to EF hands 1, 3 and 4 [109-111]. Unlike in the solution structure, CaBP1 in the crystal structure was found to be oligomerized and there were some differences in the N-lobe between the two structures. The C-terminal but not the N-terminal lobe binds to the InsP_3 receptor [110] and the binding interaction and inhibition of receptor function have been shown to require hydrophobic residues that are exposed in the Ca^{2+} -bound form of the C-lobe and that permit binding to complementary hydrophobic regions on the receptor [112]. In addition to characterising the binding interaction this structural study also suggested a mechanism whereby CaBP1 inhibited InsP_3 receptor gating by limiting inter-subunit movements to stabilise a closed state of the channel.

CaBP7 and CaBP8 (also known as calneuron II and I respectively [104, 113]) are evolutionarily distinct from the other CaBPs [103] and have a unique C-terminal transmembrane domain [114-116]. Presently, they have only one known target, phosphatidylinositol-4-kinase III β (PI4KIII β), which is also regulated by NCS-1 but is not known to be regulated by other Ca^{2+} sensors. Whereas NCS-1 stimulates the kinase at elevated $[\text{Ca}^{2+}]_i$ [117] CaBP7 and 8 inhibit its activity at resting $[\text{Ca}^{2+}]_i$ and this action can fully explain the physiological effects of CaBP7 and 8 on membrane traffic [118]. The active EF-hands in CaBP7 and 8 are present in the N-terminal domain (Fig. 4) and this domain from CaBP7 can independently bind PI4KIII β [119]. The overall tertiary structure of the Ca^{2+} -bound N-terminal domain (residues 1-100) of CaBP7 was solved by solution NMR [119]. The structure is most similar to that of the C-terminal lobes of calmodulin and CaBP1 (Fig. 5). Clues to the basis for specificity of target binding come from examination of space-filling representations of the three structures. CaBP7 has an exposed hydrophobic face that is larger in area than that found in calmodulin or CaBP1 with the presence of fewer charged residues [119]. Notably, CaBP7 lacks three of the four methionine residues in the hydrophobic pocket that are present in both the calmodulin N- and C-terminal lobes and that are required for target-binding by calmodulin [24, 120, 121]. Three of these methionines are conserved in the CaBP1 C-terminal lobe and interestingly two of these (M164 and M165) make contact with the N-terminal domain of the InsP_3 receptor in the docked complex [112]. It may be significant that the methionines in CaBP7 and also CaBP8 have been exchanged

for conformationally immobile leucine and isoleucine residues which might help explain the restricted number of targets available for interaction with CaBP7. The underlying hypothesis is that the CaBP hydrophobic binding surface can be made more flexible by increasing the number of methionines present or more rigid by reducing their number and that the degree of flexibility governs target promiscuity. Consistent with this idea calmodulin has many more known interaction partners (>300 [24]) than CaBP1 which in turn exhibits a considerably larger interactome (at least 14 targets) than CaBP7 which is currently known to interact only with PI4KIII β . It should be noted, however, that we cannot rule out that further work will identify additional targets for CaBP7 and 8.

4. Case study 3 - Regulation of voltage-gated Ca²⁺ channels by multiple Ca²⁺ sensors

Ca²⁺-entry through voltage-gated Ca²⁺ channels in neurons triggers neurotransmitter release, changes in gene expression, underpins synaptic plasticity and affects neuronal development [122-124]. The multiple types of VGCCs have been well characterised and found to undergo extensive regulation including feedback by Ca²⁺ itself leading to rapid Ca²⁺-dependent facilitation (CDF) or Ca²⁺-dependent inhibition (CDI) on a millisecond timescale. The speed of this regulation is explained by the response of the channels being due to nanodomains of Ca²⁺ near the mouth of the channel [125]. Both CDF and CDI are believed to contribute to synaptic plasticity [126-128]. A recent review has summarised the history of the discovery of VGCC regulation by Ca²⁺ and progress on the characterisation of the mechanisms particularly those involving calmodulin [129]. Regulation of VGCCs by other Ca²⁺ sensors has also been the subject of a recent review [128]. Pioneering studies identified calmodulin as a Ca²⁺ sensor required for CDI of L-type (Ca_v1.2) Ca²⁺ channels [130, 131]. It was subsequently demonstrated that Ca²⁺-free (apo) calmodulin was pre-bound to the pore-forming α_1 channel subunit and that this was mediated through the so-called IQ domain (Fig 6) of the α_1 subunit [132-134]. The fact that calmodulin is already bound to the channel subunit explains how the channels can be regulated so rapidly in response to Ca²⁺ elevation in local nanodomains.

A role for calmodulin was subsequently extended to other VGCC types. Use of Ca²⁺-insensitive calmodulin mutants with inactivated EF-hands demonstrated its requirement. Calmodulin has independent N- and C-terminal lobes which each possess 2 active EF-hands. The two lobes can show major structural movement relative to each other on ligand binding allowing them to both bind to the same helix (Fig 7). For Ca_v1.2 channels CDI was mediated through the C-lobe [130]. In contrast for Ca_v2.1 [135, 136], Ca_v2.2 [137] and

Ca_v2.3 [137] it is the N-lobe that controls CDI. Of particular interest is the finding that for P/Q-type (Ca_v2.1) channels calmodulin is required for both CDI and CDF with the N-lobe of calmodulin involved in CDI and the C-lobe in CDF [135, 136]. The majority of studies have focused on the IQ domain in the C-terminal cytoplasmic region of the subunit (Fig 6) as the binding site for calmodulin on the various channel types [138, 139]. Other studies have implicated an additional calmodulin-binding domain (CBD) in the C-terminus present in Ca_v2.1 [136] and there are likely to be additional binding sites in the N-terminal domain of the α_1 subunit (Fig 6).

The picture of Ca²⁺-dependent regulation of VGCCs became more complex when it was discovered that various other Ca²⁺ sensors could exert differing effects on CDI, CDF (Table 2) or channel current, in some cases apparently also through binding to the IQ domain [128]. The first such indication of an additional regulatory mechanism came from the finding that CaBP1 could elicit CDI itself and also reduce CDF due to calmodulin in Ca_v2.1 channels [140]. CaBP1, as described above, has been shown to have a bi-lobe structure like calmodulin [109] and it has been suggested that it exerts its effect on CDF by competing-off calmodulin from the IQ domain [109, 141, 142].

Ca_v2.1 channels appear to be under extensive regulatory control by multiple Ca²⁺ sensors. In addition to calmodulin and CaBP1 these channels are also regulated by the NCS proteins VILIP-2 and NCS-1. VILIP-2 inhibits the inactivation brought about by calmodulin and also enhances facilitation in the same way as calmodulin [143, 144] through interaction with the IQ and the CBD domains. Electrophysiological experiments implicated NCS-1 in CDF of Ca_v2.1 channels [145] and also in an autocrine pathway that negatively regulates these P/Q-type Ca²⁺ channels in adrenal chromaffin cells [146, 147]. In addition, functional effects of loss of NCS-1 (Frq) in *Drosophila* have been investigated using genetic approaches and attributed to regulation of cacophony [56, 148] which encodes an α_1 -subunit of a fly VGCC. Despite these studies a direct interaction of NCS-1 with the Ca_v2.1 channel or any other VGCC had not been reported. Recently, however, use of multiple biochemical and structural approaches has consistently demonstrated a direct interaction of NCS-1 with the Ca_v2.1 channel α_1 -subunit that requires the IQ domain but does not appear to involve the CBD in the C-terminus of the α_1 -subunit [149]. NMR analysis also demonstrated direct binding of an IQ domain peptide to NCS-1.

Members of the CaBP family have been found to regulate a range of VGCC types (Table 2). For example CaBP1 prolongs Ca_v1.2 currents and inhibits CDI [150, 151]. The gene encoding CaBP1 also gives rise to a longer isoform, caldendrin, which may be the predominant form in brain [152, 153]. Caldendrin also reduces CDI of Ca_v1.2 channels but

to a lesser extent than CaBP1 [150]. Studies of CaBP5 function in the retina have suggested that it acts as an inhibitor of CDI of Ca_v1.2 channels [154]. Potentially other cell-specific forms of regulation by multiple CaBPs of Ca_v1.3 channels have also been discovered in auditory hair cells [155-157] although CaBP1 may be the principal regulator [128]. In photoreceptors there is a potential function for CaBP4 in the regulation of Ca_v1.3 channels [158]. CaBP4 may also be important in photoreceptors for the regulation of Ca_v1.4 channels through an effect on the voltage activation of these channels [159, 160].

Many of the studies on the regulation of CDF or CDI of VGCCs by Ca²⁺ sensors have been based on expression of the sensors and VGCC subunits in heterologous cell systems. This, therefore, raises questions about the physiological relevance of the interactions of the Ca²⁺ sensors for neuronal function. Support for a significant role has come from a study in which expression of modified Ca_v1.2 channels in superior cervical ganglion (SCG) neurons was used to probe the general role for interaction of Ca²⁺ sensors in synaptic plasticity. In this study it was shown that mutation of the IQ (IM in Ca_v2.1) motif or the CBD that would prevent CDF or CDI reduced facilitation or short-term synaptic depression respectively [161]. In addition, another study using SCG neurons showed that expression of CaBP1 or VILIP-2 had effects on synaptic plasticity [127] consistent with their effects on CDF and CDI in heterologous models [162].

One problem with the originally proposed competitive model of action for CaBPs in which they need to compete-off endogenous calmodulin from the IQ domain is that calmodulin is expressed in brain at considerably higher levels than CaBPs. CaBP1 has been shown, however, to interact with an additional site in the N-terminus of Ca_v1.2 [163]. More recently it has been suggested that apo-CaBP4 and apo-calmodulin may both be associated with Ca_v1.3 channels as a consequence of CaBP4 binding to regions other than the IQ domain [156] so that the higher concentration of calmodulin would not be a significant barrier to a physiological role for CaBPs. In addition to these considerations it should also be emphasised that calmodulin interacts with a large number of cellular targets. Its availability for any given interaction is therefore difficult to accurately determine and this also likely impacts on the ability of other calcium sensors to modulate shared targets.

Findings consistent with an important role for CaBP4 in the regulation of phototransduction has come from the study of a CaBP4 mouse knock-out and also the presence of mutations in human CaBP4 [164, 165] and in Ca_v1.4 [166] that cause retinal disorders including congenital night blindness. Data from the CaBP5 knock out mouse supports a role for CaBP5 in altering retinal sensitivity though its effects on Ca_v1.2 channels [154]. In addition, a mutation in CaBP2 resulting in its truncation has been found that results in autosomal

hearing impairment. CaBP2 is expressed in cochlear hair cells where the effect of the truncation may be due to impaired suppression of CDI of Ca_v1.3 channels [167].

The structural basis for how calmodulin or other Ca²⁺ sensors exert their Ca²⁺-dependent effects on VGCCs has been a subject of intensive study. Key issues to be resolved include: 1) how can the two lobes of calmodulin have divergent functions when both bind to the IQ domain; 2) why does the C-lobe dominate in CDI in Ca_v1.2 channels but the N-lobe does so in Ca_v2 channels; 3) how can binding of calmodulin to an intracellular region of the channel α_1 subunit affect the gating properties of the channel pore; 4) how can additional Ca²⁺ sensors that mediate differing effects do so particularly in the presence of calmodulin tightly bound to the IQ domain [129, 168]. A major focus for the calmodulin control of VGCCs has been in analysis of the IQ domain interaction. Initial studies characterised the structure of the complex of Ca²⁺-bound calmodulin with an IQ-like peptide from Ca_v1.2 (Fig 7). These indicated that calmodulin wrapped around the IQ helix with both the N- and C-terminal lobes binding and with the helix bound in an unusual parallel manner with the N- and C-lobes of calmodulin binding to the N- and C-terminal ends of the IQ helix [169, 170]. It was subsequently found that calmodulin bound the IQ peptides of Ca_v2.1, Ca_v2.2 or Ca_v2.3 channels in an anti-parallel manner giving a possible explanation for the reverse role of the N- and C-lobes in CDI of the two classes of channels [138]. This explanation was not supported by another study, however, that showed that all IQ/calmodulin interactions had a parallel conformation although this study was based on complexes with a shorter IQ peptide [139]. More recent studies have begun to explore the structural basis for calmodulin interaction with larger constructs from the C-terminal domain of the VGCCs (reviewed in [168]). It is clear that much still remains to be learnt but recent work has suggested that interactions in the C-terminus outside the IQ domain and with the N-terminus of the α_1 subunit involving the Ca²⁺-loaded N-lobe may be important [171].

A full structural characterisation of the interaction of CaBP1 with VGCCs is not available but one analysis has used chimeras with calmodulin and specific mutants to dissect the requirements for regulation of Ca_v1.2 channels. This has demonstrated that both lobes of CaBP1 are functionally important with the C-lobe anchoring to the channel subunit as does the C-lobe of calmodulin. The N-lobe and also the inter-lobe linker specific for the CaBPs are required for modification of channel function and this could contribute to the different effects on the channel of CaBP1 compared to calmodulin [109]. It has been suggested that differential effects of calmodulin and CaBP1 may in part be due to the structurally different interactions with the IQ domain [109]. It is also possible that interactions with other regions of the α_1 subunit may be important but this will require further structural characterisation. There

is currently no published data on the structural bases for the interactions of other Ca^{2+} sensors with VGCCs.

5. Conclusions

One aspect that emerges from consideration of the three case studies above is the variation in level of specificity versus promiscuity of the Ca^{2+} sensors which is determined by fundamental structural characteristics of the proteins. Calmodulin is the most promiscuous with many target proteins. It consists of two independent lobes joined by a flexible linker that allows calmodulin to wrap around and interact with target proteins in multiple different ways using mutually induced fit to ensure high affinity interaction [172]. Also calmodulin possesses several residues with flexible side chains on its hydrophobic binding surface that also seems to be important to its promiscuity accommodating binding of numerous different ligands to the same protein surface. The CaBP proteins while superficially similar to calmodulin in having two N- and C-terminal lobes have a reduced range of targets as a consequence of having one or more inactivated EF hands and a reduction to varying levels (see CaBP1 versus CaBP7/8) in the number of methionines presented on their binding surface. They also have specialisations (such as myristoylation or the transmembrane domains of CaBP7 and 8) that will limit their access to potential cytoplasmic target proteins.

On Ca^{2+} binding, Ca^{2+} sensors undergo a conformational change that allows them to bind ligands and may then undergo further conformational changes. This in turn will allow them to regulate the function of the target proteins. Most of the available structural characterisation has provided information on the binding mechanism and specificity but so far has provided little information on how the activity of the target protein is regulated. This is particularly notable in the case of VGCCs where even different lobes of calmodulin can have different functional effects after binding to a site on the channel α_1 subunit some distance from the channel pore. For VGCC regulation we need further information on the structural basis of interactions of Ca^{2+} sensors outside of the IQ domain, with the rest of the C-terminus, with sites in the N-terminus of the α_1 subunit and also potentially with other subunits of the holo-channel. The NCS proteins such as NCS-1 have a smaller range of targets and differ from calmodulin in having two lobes that have a very rigid inter-relationship severely reducing the conformational flexibility between the two lobes. Target binding is due to the exposure of a hydrophobic groove the size and surrounding residues of which in conjunction with a variable and more flexible C-terminal tail determine which ligands can bind. A completely uninvestigated aspect of NCS-target interaction is how NCS protein binding influences target

conformation, structure and how this translates to function. Again, structural insights in this regard are limited by the complexes characterised to date containing only short peptides derived from the target protein. Further progress in this field will, therefore, require characterisation of Ca^{2+} sensors complexes with larger, ideally full-length, target proteins in order to contribute to an understanding of the mechanistic basis of differential signal processing in response to Ca^{2+} signals.

Acknowledgements

Work in the authors' laboratory was supported by the Wellcome Trust.

References

- [1] M. Brini, T. Cali, D. Ottolini, E. Carafoli, Neuronal calcium signaling: function and dysfunction, *Cell Mol Life Sci*, 71 (2014) 2787-2814.
- [2] M.J. Berridge, Neuronal calcium signalling, *Neuron*, 21 (1998) 13-26.
- [3] M.J. Berridge, Calcium hypothesis of Alzheimer's disease, *Pflugers Arch*, 459 (2010) 441-449.
- [4] G.E. Stutzmann, A. Caccamo, F.M. LaFerla, I. Parker, Dysregulated IP3 signaling in cortical neurons of knock-in mice expressing an Alzheimer's-linked mutation in presenilin1 results in exaggerated Ca^{2+} signals and altered membrane excitability, *J Neurosci*, 24 (2004) 508-513.
- [5] E. Popugaeva, I. Bezprozvanny, Role of endoplasmic reticulum Ca^{2+} signaling in the pathogenesis of Alzheimer disease, *Front Mol Neurosci*, 6 (2013) 29.
- [6] M.J. Berridge, Calcium signalling and psychiatric disease: bipolar disorder and schizophrenia, *Cell Tissue Res*, (2014).
- [7] C. Cross-Disorder Group of the Psychiatric Genomics, Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis, *Lancet*, 381 (2013) 1371-1379.
- [8] M.J. Berridge, P. Lipp, M.D. Bootman, The versatility and universality of calcium signalling., *Nature Rev. Mol. Cell Biol.*, 1 (2000) 11-21.
- [9] H. Bading, Nuclear calcium signalling in the regulation of brain function, *Nat Rev Neurosci*, 14 (2013) 593-608.
- [10] H. Takechi, J. Eilers, A. Konnerth, A new class of synaptic response involving calcium release in dendritic spines, *Nature*, 396 (1998) 757-760.

- [11] M. Segal, E. Korkotian, Endoplasmic reticulum calcium stores in dendritic spines, *Front Neuroanat*, 8 (2014) 64.
- [12] J.I. Morgan, T. Curran, Role of ion flux in the control of c-fos expression, *Nature*, 322 (1986) 552-555.
- [13] H. Bito, K. Deisseroth, R.W. Tsien, Ca^{2+} -dependent regulation in neuronal gene expression, *Current Opinion in Neurobiology*, 7 (1997) 419-429.
- [14] S.-N. Yang, Y.-G. Tang, R.S. Zucker, Selective induction of LTP and LTD by postsynaptic $[\text{Ca}^{2+}]_i$ elevation., *J. Neurophysiol.*, 81 (1999) 781-787.
- [15] N.C. Spitzer, N.J. Lautermilch, R.D. Smith, T.M. Gomez, Coding of neuronal differentiation by calcium transients, *BioEssays*, 22 (2000) 811-817.
- [16] P.K. Dash, A.N. Moore, N. Kobori, J.D. Runyan, Molecular activity underlying working memory, *Learn Mem*, 14 (2007) 554-563.
- [17] H. Bading, D.D. Ginty, M.E. Greenberg, Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways, *Science*, 260 (1993) 181-186.
- [18] K. Deisseroth, E.K. Heist, R.W. Tsien, Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons, *Nature*, 392 (1998) 198-202.
- [19] D.G. Wheeler, R.D. Groth, H. Ma, C.F. Barrett, S.F. Owen, P. Safa, R.W. Tsien, $\text{CaV}1$ and $\text{CaV}2$ channels engage distinct modes of Ca^{2+} signaling to control CREB-dependent gene expression, *Cell*, 149 (2012) 1112-1124.
- [20] H.V. McCue, L.P. Haynes, R.D. Burgoyne, The Diversity of Calcium Sensor Proteins in the Regulation of Neuronal Function, *Cold Spring Harb Perspect Biol*, 2 (2010) a004085.
- [21] R. Fernandez-Chacon, A. Konigstorfer, S.H. Gerber, J. Garcia, M.F. Matos, C.F. Stevens, N. Brose, J. Rizo, C. Rosenmund, T.C. Sudhof, Synaptotagmin I functions as a calcium regulator of release probability, *Nature*, 410 (2001) 41-49.
- [22] V. Gerke, C.E. Creutz, S.E. Moss, Annexins: linking Ca^{2+} signalling to membrane dynamics, *Nat Rev Mol Cell Biol*, 6 (2005) 449-461.
- [23] N.D. Moncreif, R.H. Kretsinger, M. Goodman, Evolution of EF-hand calcium-modulated proteins. 1. Relationships based on amino-acid sequences., *J Mol Evolution*, 30 (1990) 522-562.
- [24] M. Ikura, J.B. Ames, Genetic polymorphism and protein conformational plasticity in the calmodulin superfamily: two ways to promote multifunctionality, *Proc. Natl. Acad. Sci. U.S.A.*, 103 (2006) 1159-1164.
- [25] T. Yamauchi, Neuronal Ca^{2+} /calmodulin-dependent protein kinase II--discovery, progress in a quarter of a century, and perspective: implication for learning and memory, *Biol Pharm Bull*, 28 (2005) 1342-1354.
- [26] F. Haeseleer, I. Sokal, C.L.M.J. Verlinde, H. Erdjument-Bromage, P. Tempst, A.N. Pronin, J.L. Benovic, R.N. Fariss, K. Palczewski, Five members of a novel Ca^{2+} binding

- protein (CABP) subfamily with similarity to calmodulin, *J. Biol. Chem.*, 275 (2000) 1247-1260.
- [27] L.P. Haynes, H.V. McCue, R.D. Burgoyne, Evolution and functional diversity of the Calcium Binding Proteins (CaBPs), *Front Mol Neurosci*, 5 (2012) 9.
- [28] R.D. Burgoyne, J.L. Weiss, The neuronal calcium sensor family of Ca^{2+} -binding proteins., *Biochem. J.*, 353 (2001) 1-12.
- [29] R.D. Burgoyne, Neuronal calcium sensor proteins: generating diversity in neuronal Ca^{2+} signalling, *Nat Rev Neurosci*, 8 (2007) 182-193.
- [30] R.D. Burgoyne, L.P. Haynes, Understanding the physiological roles of the neuronal calcium sensor proteins, *Mol Brain*, 5 (2012) 2.
- [31] M. Paterlini, V. Revilla, A.L. Grant, W. Wisden, Expression of the neuronal calcium sensor protein family in the rat brain., *Neurosci.*, 99 (2000) 205-216.
- [32] V. Raghuram, Y. Sharma, M.R. Kreutz, Ca^{2+} sensor proteins in dendritic spines: a race for Ca^{2+} , *Front Mol Neurosci*, 5 (2012) 61.
- [33] D. Anderson, J.D. Engbers, N.C. Heath, T.M. Bartoletti, W.H. Mehafeff, G.W. Zamponi, R.W. Turner, The Cav3-Kv4 complex acts as a calcium sensor to maintain inhibitory charge transfer during extracellular calcium fluctuations, *J Neurosci*, 33 (2013) 7811-7824.
- [34] M.F. Navedo, J.W. Hell, AKAP5 keeps L-type channels and NFAT on their toes, *Cell Reports*, 7 (2014) 1341-1342.
- [35] M. Kitazawa, Y. Kubo, K. Nakajo, The Stoichiometry and Biophysical Properties of the Kv4 Potassium Channel Complex with K^+ Channel-interacting Protein (KChIP) Subunits Are Variable, Depending on the Relative Expression Level, *J Biol Chem*, 289 (2014) 17597-17609.
- [36] K. Kunjilwar, Y. Qian, P.J. Pfaffinger, Functional stoichiometry underlying KChIP regulation of Kv4.2 functional expression, *J Neurochem*, 126 (2013) 462-472.
- [37] J.B. Ames, S. Lim, Molecular structure and target recognition of neuronal calcium sensor proteins, *Biochim Biophys Acta*, 1820 (2012) 1205-1213.
- [38] J.B. Ames, R. Ishima, T. Tanaka, J.I. Gordon, L. Stryer, M. Ikura, Molecular mechanics of calcium-myristoyl switches., *Nature*, 389 (1997) 198-202.
- [39] D.W. O'Callaghan, A.V. Tepikin, R.D. Burgoyne, Dynamics and calcium-sensitivity of the Ca^{2+} -myristoyl switch protein hippocalcin in living cells., *J. Cell Biol.*, 163 (2003) 715-721.
- [40] O. Markova, D. Fitzgerald, A. Stepanyuk, A. Dovgan, V. Cherkas, A. Tepikin, R.D. Burgoyne, P. Belan, Hippocalcin signaling via site-specific translocation in hippocampal neurons, *Neuroscience letters*, 442 (2008) 152-157.
- [41] A.V. Dovgan, V.P. Cherkas, A.R. Stepanyuk, D.J. Fitzgerald, L.P. Haynes, A.V. Tepikin, R.D. Burgoyne, P.V. Belan, Decoding glutamate receptor activation by the Ca sensor protein hippocalcin in rat hippocampal neurons, *Eur J Neurosci*, 32 (2010) 347-358.

- [42] C.L. Palmer, W. Lim, P.G. Hastie, M. Toward, V.I. Korolchuk, S.A. Burbidge, G. Banting, G.L. Collingridge, J.T. Isaac, J.M. Henley, Hippocalcin functions as a calcium sensor in hippocampal LTD, *Neuron*, 47 (2005) 487-494.
- [43] J. Jo, G.H. Son, B.L. Winters, M.J. Kim, D.J. Whitcomb, B.A. Dickinson, Y.B. Lee, K. Futai, M. Amici, M. Sheng, G.L. Collingridge, K. Cho, Muscarinic receptors induce LTD of NMDAR EPSCs via a mechanism involving hippocalcin, AP2 and PSD-95, *Nat Neurosci*, 13 (2010) 1216-1224.
- [44] A.V. Tzingounis, M. Kobayashi, K. Takamatsu, R.A. Nicoll, Hippocalcin gates the calcium activation of the slow afterhyperpolarization in hippocampal pyramidal cells, *Neuron*, 53 (2007) 487-493.
- [45] C. Spilker, T. Dresbach, K.-H. Braunewell, Reversible translocation and activity-dependent localisation of the calcium-myristoyl switch protein VILIP-1 to different membrane compartments in living hippocampal neurons., *J. Neurosci.*, 22 (2002).
- [46] K.H. Braunewell, A.J. Klein-Szanto, Visinin-like proteins (VSNLs): interaction partners and emerging functions in signal transduction of a subfamily of neuronal Ca^{2+} -sensor proteins, *Cell Tissue Res*, 335 (2009) 301-316.
- [47] W.F. An, M.R. Bowlby, M. Bett, J. Cao, H.P. Ling, G. Mendoza, J.W. Hinson, K.I. Mattsson, B.W. Strassle, J.S. Trimmer, K.J. Rhodes, Modulation of A-type potassium channels by a family of calcium sensors, *Nature*, 403 (2000) 553-556.
- [48] B. Hasdemir, D.J. Fitzgerald, I.A. Prior, A.V. Tepikin, R.D. Burgoyne, Traffic of Kv4 K^{+} channels mediated by KChIP1 is via a novel post-ER vesicular pathway., *J Cell Biol*, 171 (2005) 459-469.
- [49] K.J. Rhodes, K.I. Carroll, M.A. Sung, L.C. Doliveira, M.M. Monaghan, S.L. Burke, B.W. Strassle, L. Buchwalder, M. Menegola, J. Cao, F.W. An, J.S. Trimmer, KChIPs and Kv4 α subunits as integral components of A-type potassium channels in mammalian brain, *J. Neurosci.*, 24 (2004) 7903-7915.
- [50] J. Dabrowska, D.G. Rainnie, Expression and distribution of Kv4 potassium channel subunits and potassium channel interacting proteins in subpopulations of interneurons in the basolateral amygdala, *Neuroscience*, 171 (2010) 721-733.
- [51] J.D. Buxbaum, E.K. Choi, Y.X. Luo, C. Lilliehook, A.C. Crowley, D.E. Merriam, W. Wasco, Calsenilin: A calcium-binding protein that interacts with the presenilins and regulates the levels of a presenilin fragment, *Nature Medicine*, 4 (1998) 1177-1181.
- [52] A.M. Carrion, W.A. Link, F. Ledo, B. Mellstrom, J.R. Naranjo, DREAM is a Ca^{2+} -regulated transcriptional repressor., *Nature*, 398 (1999) 80-84.
- [53] W.A. Link, F. Ledo, B. Torres, M. Palczewski, T.M. Madsen, M. Savignac, J.P. Albar, B. Mellstrom, J.R. Naranjo, Day-night changes in downstream regulatory element antagonist

- modulator/potassium channel interacting protein activity contribute to circadian gene expression in pineal gland., *J Neurosci*, 24 (2004) 5346-5355.
- [54] N. Venn, L.P. Haynes, R.D. Burgoyne, Specific effects of KChIP3/calsenilin/DREAM but not KChIPs1, 2 and 4 on calcium signalling and regulated secretion in PC12 cells, *Biochem J*, 413 (2008) 71-80.
- [55] P. Gierke, C. Zhao, M. Brackmann, B. Linke, U. Heinemann, K.-H. Braunewell, Expression analysis of members of the neuronal calcium sensor protein family: combining bioinformatics and Western Blot analysis, *Biochem. Biophys. Res. Comm.*, 323 (2004) 38-43.
- [56] J.S. Dason, J. Romero-Pozuelo, H.L. Atwood, A. Ferrus, Multiple roles for frequenin/NCS-1 in synaptic function and development, *Mol Neurobiol*, 45 (2012) 388-402.
- [57] M. Mikhaylova, J. Hradsky, M.R. Kreutz, Between promiscuity and specificity: novel roles of EF-hand calcium sensors in neuronal Ca^{2+} signalling, *J Neurochem*, 118 (2011) 695-713.
- [58] T.L. Kerrigan, J.W. Daniel, P.L. Regan, K. Cho, The role of neuronal calcium sensors in balancing synaptic plasticity and synaptic dysfunction, *Front Mol Neurosci*, 5 (2012) 57.
- [59] M. Gomez, E. De Castro, E. Guarin, H. Sasakura, A. Kuhara, I. Mori, T. Bartfai, C.I. Bargmann, P. Nef, Ca^{2+} signalling via the neuronal calcium sensor-1 regulates associative learning and memory in *C. elegans*., *Neuron*, 30 (2001) 241-248.
- [60] V.M. Martin, J.R. Johnson, L.P. Haynes, J.W. Barclay, R.D. Burgoyne, Identification of key structural elements for neuronal calcium sensor-1 function in the regulation of the temperature-dependency of locomotion in *C. elegans*, *Mol Brain*, 6 (2013) 39.
- [61] D. Wang, D. O'Halloran, M.B. Goodman, GCY-8, PDE-2, and NCS-1 are critical elements of the cGMP-dependent thermotransduction cascade in the AFD neurons responsible for *C. elegans* thermotaxis, *J Gen Physiol*, 142 (2013) 437-449.
- [62] B. Blasiole, N. Kabbani, W. Boehmler, B. Thisse, C. Thisse, V. Canfield, R. Levenson, Neuronal calcium sensor-1 gene ncs-1 is essential for semicircular canal formation in zebrafish inner ear., *J. Neurobiol.*, 64 (2005) 285-297.
- [63] O. Pongs, J. Lindemeier, X.R. Zhu, T. Theil, D. Endelkamp, I. Krah-Jentgens, H.-G. Lambrecht, K.W. Koch, J. Schwemer, R. Rivosecchi, A. Mallart, J. Galceran, I. Canal, J.A. Barbas, A. Ferrus, Frequenin - A novel calcium-binding protein that modulates synaptic efficacy in the drosophila nervous system., *Neuron*, 11 (1993) 15-28.
- [64] K.B. Hendricks, B.Q. Wang, E.A. Schnieders, J. Thorner, Yeast homologue of neuronal frequenin is a regulator of phosphatidylinositol-4-OH kinase., *Nature Cell Biology*, 1 (1999) 234-241.

- [65] B.W. McFerran, M.E. Graham, R.D. Burgoyne, NCS-1, the mammalian homologue of frequenin is expressed in chromaffin and PC12 cells and regulates neurosecretion from dense-core granules., *J Biol Chem*, 273 (1998) 22768-22772.
- [66] J. Jo, S. Heon, M.J. Kim, G.H. Son, Y. Park, J.M. Henley, J.L. Weiss, M. Sheng, G.L. Collingridge, K. Cho, Metabotropic glutamate receptor-mediated LTD involves two interacting Ca^{2+} sensors, NCS-1 and PICK1, *Neuron*, 60 (2008) 1095-1111.
- [67] P.K. Yip, L.F. Wong, T.A. Sears, R.J. Yanez-Munoz, S.B. McMahon, Cortical overexpression of neuronal calcium sensor-1 induces functional plasticity in spinal cord following unilateral pyramidal tract injury in rat, *PLoS Biol*, 8 (2010) e1000399.
- [68] J.L. Weiss, H. Hui, R.D. Burgoyne, Neuronal calcium sensor-1 regulation of calcium channels, secretion, and neuronal outgrowth, *Cell Mol Neurobiol*, 30 (2010) 1283-1292.
- [69] V.B. de Rezende, D.V. Rosa, C.M. Comim, L.A. Magno, A.L. Rodrigues, P. Vidigal, A. Jeromin, J. Quevedo, M.A. Romano-Silva, NCS-1 deficiency causes anxiety and depressive-like behavior with impaired non-aversive memory in mice, *Physiol, Behav.*, 130 (2014) 91-98.
- [70] B.J. Saab, J. Georgiou, A. Nath, F.J. Lee, M. Wang, A. Michalon, F. Liu, I.M. Mansuy, J.C. Roder, NCS-1 in the dentate gyrus promotes exploration, synaptic plasticity, and rapid acquisition of spatial memory, *Neuron*, 63 (2009) 643-656.
- [71] L.P. Haynes, D.J. Fitzgerald, B. Wareing, D.W. O'Callaghan, A. Morgan, R.D. Burgoyne, Analysis of the interacting partners of the neuronal calcium-binding proteins L-CaBP1, hippocalcin, NCS-1 and neurocalcin., *Proteomics*, 6 (2006) 1822-1832.
- [72] N.C. Schaad, E. De Castro, S. Nef, S. Hegi, R. Hinrichsen, M.E. Martone, M.H. Ellisman, R. Sikkink, J. Sygush, P. Nef, Direct modulation of calmodulin targets by the neuronal calcium sensor NCS-1., *Proc. Natl. Acad. Sci. USA*, 93 (1996) 9253-9258.
- [73] D.J. Fitzgerald, R.D. Burgoyne, L.P. Haynes, Neuronal calcium sensor proteins are unable to modulate NFAT activation in mammalian cells, *Biochim Biophys Acta*, 1780 (2008) 240-248.
- [74] N. Kabbani, L. Negyessy, R. Lin, P. Goldman-Rakic, R. Levenson, Interaction with the neuronal calcium sensor NCS-1 mediates desensitization of the D2 dopamine receptor., *J. Neurosci.*, 22 (2002) 8476-8486.
- [75] A. Dagher, T.W. Robbins, Personality, addiction, dopamine: insights from Parkinson's disease, *Neuron*, 61 (2009) 502-510.
- [76] G.F. Koob, The neurobiology of addiction: a neuroadaptational view relevant for diagnosis, *Addiction*, 101 Suppl 1 (2006) 23-30.
- [77] P. Seeman, Dopamine receptor sequences. Therapeutic levels of neuroleptics occupy D2 receptors, clozapine occupies D4, *Neuropsychopharmacology*, 7 (1992) 261-284.

- [78] P.O. Koh, A.S. Undie, N. Kabbani, R. Levenson, P.S. Goldman-Rakic, M.S. Lidow, Up-regulation of neuronal calcium sensor-1 (NCS-1) in the prefrontal cortex of schizophrenic and bipolar patients., *Proc. Natl. Acad. Sci. U.S.A.*, 100 (2003) 313-317.
- [79] N. Kabbani, R. Levenson, Antipsychotic-induced alterations in D2 dopamine receptor interacting proteins within the cortex, *Neuroreport*, 17 (2006) 299-301.
- [80] P.K. Multani, T.K. Clarke, S. Narasimhan, L. Ambrose-Lanci, K.M. Kampman, H.M. Pettinati, D.W. Oslin, C.P. O'Brien, W.H. Berrettini, F.W. Lohoff, Neuronal calcium sensor-1 and cocaine addiction: A genetic association study in African-Americans and European Americans, *Neuroscience letters*, 531 (2012) 46-51.
- [81] M.C. Ritz, R.J. Lamb, S.R. Goldberg, M.J. Kuhar, Cocaine receptors on dopamine transporters are related to self-administration of cocaine, *Science*, 237 (1987) 1219-1223.
- [82] E. Dragicevic, C. Poetschke, J. Duda, F. Schlaudraff, S. Lammel, J. Schiemann, M. Fauler, A. Hetzel, M. Watanabe, R. Lujan, R.C. Malenka, J. Striessnig, B. Liss, Cav1.3 channels control D2-autoreceptor responses via NCS-1 in substantia nigra dopamine neurons, *Brain*, 137 (2014) 2287-2302.
- [83] L.Y. Lian, S.R. Pandalaneni, P. Patel, H.V. McCue, L.P. Haynes, R.D. Burgoyne, Characterisation of the Interaction of the C-Terminus of the Dopamine D2 Receptor with Neuronal Calcium Sensor-1, *PLoS ONE*, 6 (2011) e27779.
- [84] N. Bahi, G. Friocourt, A. Carrié, M.E. Graham, J.L. Weiss, P. Chafey, F. Fauchereau, R.D. Burgoyne, J. Chelly, IL1 receptor accessory protein like, a protein involved in X-linked mental retardation, interacts with Neuronal Calcium Sensor-1 and regulates exocytosis., *Human Mol. Genetics*, 12 (2003) 1415-1425.
- [85] Y.H. Zhang, B.L. Huang, K.K. Niakan, L.L. McCabe, E.R. McCabe, K.M. Dipple, IL1RAPL1 is associated with mental retardation in patients with complex glycerol kinase deficiency who have deletions extending telomeric of DAX1, *Hum Mutat*, 24 (2004) 273.
- [86] E. Tabolacci, M.G. Pomponi, R. Pietrobono, A. Terracciano, P. Chiurazzi, G. Neri, A truncating mutation in the IL1RAPL1 gene is responsible for X-linked mental retardation in the MRX21 family, *Am J Med Genet A*, 140 (2006) 482-487.
- [87] A. Piton, J.L. Michaud, H. Peng, S. Aradhya, J. Gauthier, L. Mottron, N. Champagne, R.G. Lafreniere, F.F. Hamdan, R. Joober, E. Fombonne, C. Marineau, P. Cossette, M.P. Dube, P. Haghighi, P. Drapeau, P.A. Barker, S. Carbonetto, G.A. Rouleau, Mutations in the calcium-related gene IL1RAPL1 are associated with autism, *Hum Mol Genet*, 17 (2008) 3965-3974.
- [88] M.T. Handley, L.Y. Lian, L.P. Haynes, R.D. Burgoyne, Structural and functional deficits in a neuronal calcium sensor-1 mutant identified in a case of autistic spectrum disorder, *PLoS ONE*, 5 (2010) e10534.

- [89] D.W. O'Callaghan, L. Ivings, J.L. Weiss, M.C. Ashby, A.V. Tepikin, R.D. Burgoyne, Differential use of myristoyl groups on neuronal calcium sensor proteins as a determinant of spatio-temporal aspects of Ca^{2+} -signal transduction., *J Biol Chem*, 277 (2002) 14227-14237.
- [90] D.W. O'Callaghan, B. Hasdemir, M. Leighton, R.D. Burgoyne, Residues within the myristoylation motif determine intracellular targeting of the neuronal Ca^{2+} sensor protein KChIP1 to post-ER transport vesicles and traffic of Kv4 K⁺ channels, *J. Cell Sci.*, 116 (2003) 4833-4845.
- [91] S. Lim, T. Strahl, J. Thorner, J.B. Ames, Structure of a Ca^{2+} -myristoyl switch protein that controls activation of a phosphatidylinositol 4-kinase in fission yeast, *J Biol Chem*, 286 (2011) 12565-12577.
- [92] M. Pioletti, F. Findeisen, G.L. Hura, D.L. Minor, Three-dimensional structure of the KChIP1–Kv4.3 T1 complex reveals a cross-shaped octamer, *Nature Struct. Mol. Biol.*, 13 (2006) 987-995.
- [93] H. Wang, Y. Yan, Q. Liu, Y. Huang, Y. Shen, L. Chen, Y. Chen, Q. Yang, Q. Hao, K. Wang, J. Chai, Structural basis for modulation of Kv4 K⁺ channels by auxiliary KChIP subunits, *Nat Neurosci*, 10 (2007) 32-39.
- [94] J.B. Ames, K. Levay, J.N. Wingard, J.D. Lusin, V.Z. Slepak, Structural Basis for Calcium-induced Inhibition of Rhodopsin Kinase by Recoverin, *J Biol Chem*, 281 (2006) 37237-37245.
- [95] E. Zernii, K. Komolov, S. Permyakov, T. Kolpakova, D. Dell Orco, A. Poetzsch, E. Knyazeva, I. Grigoriev, E. Permyakov, I. Senin, P. Philippov, K.W. Koch, Involvement of recoverin C-terminal segment in recognition of the target enzyme rhodopsin kinase, *Biochem J*, 435 (2011) 441-450.
- [96] P.O. Heidarsson, I.J. Bjerrum-Bohr, G.A. Jensen, O. Pongs, B.E. Finn, F.M. Poulsen, B.B. Kragelund, The C-terminal tail of human neuronal calcium sensor 1 regulates the conformational stability of the Ca^{2+} -activated state, *J Mol Biol*, 417 (2012) 51-64.
- [97] Y. Bourne, J. Dannenberg, V. Pollmann, P. Marchot, O. Pongs, Immunocytochemical localisation and crystal structure of human frequenin (neuronal calcium sensor 1), *J. Biol. Chem.*, 276 (2001) 11949-11955.
- [98] T. Strahl, I.G. Huttner, J.D. Lusin, M. Osawa, D. King, J. Thorner, J.B. Ames, Structural insights into activation of phosphatidylinositol 4-kinase (pik1) by yeast frequenin (Frq1), *J Biol Chem*, 282 (2007) 30949-30959.
- [99] M.R. Edwards, J.R. Johnson, K. Rankin, R.E. Jenkins, C. Maguire, A. Morgan, R.D. Burgoyne, J.W. Barclay, PKC-2 Phosphorylation of UNC-18 Ser322 in AFD Neurons Regulates Temperature Dependency of Locomotion, *J Neurosci*, 32 (2012) 7042-7051.

- [100] A. Sanchez-Gracia, J. Romero-Pozuelo, A. Ferrus, Two frequenins in *Drosophila*: unveiling the evolutionary history of an unusual neuronal calcium sensor (NCS) duplication, *BMC evolutionary biology*, 10 (2010) 54.
- [101] J. Romero-Pozuelo, J.S. Dason, H.L. Atwood, A. Ferrus, Chronic and acute alterations in the functional levels of Frequenins 1 and 2 reveal their roles in synaptic transmission and axon terminal morphology, *Eur J Neurosci*, 26 (2007) 2428-2443.
- [102] J. Romero-Pozuelo, J.S. Dason, A. Mansilla, S. Banos-Mateos, J.L. Sardina, A. Chaves-Sanjuan, J. Jurado-Gomez, E. Santana, H.L. Atwood, A. Hernandez-Hernandez, M.J. Sanchez-Barrena, A. Ferrus, The guanine-exchange factor Ric8a binds the calcium sensor NCS-1 to regulate synapse number and probability of release, *J Cell Sci*, 127 (2014) 4246-4259.
- [103] H.V. McCue, L.P. Haynes, R.D. Burgoyne, Bioinformatic analysis of CaBP/calneuron proteins reveals a family of highly conserved vertebrate Ca^{2+} -binding proteins, *BMC Res Notes*, 3 (2010) 118.
- [104] M. Mikhaylova, Y. Sharma, C. Reissner, F. Nagel, P. Aravind, B. Rajini, K.H. Smalla, E.D. Gundelfinger, M.R. Kreutz, Neuronal Ca^{2+} signaling via caldendrin and calneurons, *Biochim Biophys Acta*, 1763 (2006) 1229-1237.
- [105] J. Yang, S. McBride, D.-O.D. Mak, N. Vardi, K. Palczewski, F. Haeseleer, J.K. Foskett, Identification of a family of calcium sensors as protein ligands of inositol trisphosphate receptor Ca^{2+} release channels., *Proc. Natl. Acad. Sci. USA*, 99 (2002) 7711-7716.
- [106] S. Patel, S.A. Morris, C.E. Adkins, G. O'Beirne, C.W. Taylor, Ca^{2+} -independent inhibition of inositol trisphosphate receptors by calmodulin: redistribution of calmodulin as a possible means of regulating Ca^{2+} mobilization, *Proc Natl Acad Sci U S A*, 94 (1997) 11627-11632.
- [107] L.P. Haynes, A.V. Tepikin, R.D. Burgoyne, Calcium Binding Protein 1 is an inhibitor of agonist-evoked, inositol 1,4,5-trisphosphate-mediated calcium signalling., *J. Biol.Chem.*, 279 (2004) 547-555.
- [108] N.N. Kasri, A.M. Holmes, G. Bultynck, J.B. Parys, M.D. Bootman, K. Rietdorf, L. Missiaen, F. McDonald, H.D. Smedt, S.J. Conway, A.B. Holmes, M.J. Berridge, H.L. Roderick, Regulation of InsP_3 receptor activity by neuronal Ca^{2+} -binding proteins., *EMBO J.*, 23 (2004) 1-10.
- [109] F. Findeisen, D.L. Minor, Jr., Structural basis for the differential effects of CaBP1 and calmodulin on $\text{Ca(V)}1.2$ calcium-dependent inactivation, *Structure*, 18 (2010) 1617-1631.
- [110] C. Li, J. Chan, F. Haeseleer, K. Mikoshiba, K. Palczewski, M. Ikura, J.B. Ames, Structural insights into Ca^{2+} -dependent regulation of inositol 1,4,5-trisphosphate receptors by CaBP1, *J Biol Chem*, 284 (2009) 2472-2481.

- [111] S. Park, C. Li, J.B. Ames, Nuclear magnetic resonance structure of calcium-binding protein 1 in a Ca^{2+} -bound closed state: Implications for target recognition, *Protein Sci*, 20 (2011) 1356-1366.
- [112] C. Li, M. Enomoto, A.M. Rossi, M.D. Seo, T. Rahman, P.B. Stathopoulos, C.W. Taylor, M. Ikura, J.B. Ames, CaBP1, a neuronal Ca^{2+} sensor protein, inhibits inositol trisphosphate receptors by clamping intersubunit interactions, *Proc Natl Acad Sci U S A*, 110 (2013) 8507-8512.
- [113] Y.Q. Wu, X. Lin, C.M. Liu, M. Jamrich, L.G. Shaffer, Identification of a human brain-specific gene, calneuron 1, a new member of the calmodulin superfamily, *Mol Genet Metab*, 72 (2001) 343-350.
- [114] H.V. McCue, R.D. Burgoyne, L.P. Haynes, Membrane targeting of the EF-hand containing calcium-sensing proteins CaBP7 and CaBP8, *Biochem Biophys Res Commun*, 380 (2009) 825-831.
- [115] H.V. McCue, R.D. Burgoyne, L.P. Haynes, Determination of the Membrane Topology of the Small EF-Hand Ca-Sensing Proteins CaBP7 and CaBP8, *PLoS ONE*, 6 (2011) e17853.
- [116] J. Hradsky, V. Raghuram, P.P. Reddy, G. Navarro, M. Hupe, V. Casado, P.J. McCormick, Y. Sharma, M.R. Kreutz, M. Mikhaylova, Post-translational membrane insertion of tail-anchored transmembrane EF-hand Ca^{2+} sensor calneurons requires the TRC40/Asna1 protein chaperone, *J Biol Chem*, 286 (2011) 36762-36776.
- [117] L.P. Haynes, M.W. Sherwood, N.J. Dolman, R.D. Burgoyne, Specificity, promiscuity and localization of ARF protein interactions with NCS-1 and phosphatidylinositol-4 kinase-III β , *Traffic*, 8 (2007) 1080-1092.
- [118] M. Mikhaylova, P.P. Reddy, T. Munsch, P. Landgraf, S.K. Suman, K.H. Smalla, E.D. Gundelfinger, Y. Sharma, M.R. Kreutz, Calneurons provide a calcium threshold for trans-Golgi network to plasma membrane trafficking, *Proc Natl Acad Sci U S A*, 106 (2009) 9093-9098.
- [119] H.V. McCue, P. Patel, A.P. Herbert, L.Y. Lian, R.D. Burgoyne, L.P. Haynes, Solution NMR Structure of the Ca^{2+} -bound N-terminal Domain of CaBP7: a regulator of Golgi trafficking, *J Biol Chem*, 287 (2012) 38231-38243.
- [120] M. Zhang, M. Li, J.H. Wang, H.J. Vogel, The effect of Met-->Leu mutations on calmodulin's ability to activate cyclic nucleotide phosphodiesterase, *J Biol Chem*, 269 (1994) 15546-15552.
- [121] K.T. O'Neil, W.F. DeGrado, How calmodulin binds its targets: sequence independent recognition of amphiphilic alpha-helices, *Trends in biochemical sciences*, 15 (1990) 59-64.
- [122] W.A. Catterall, A.P. Few, Calcium channel regulation and presynaptic plasticity, *Neuron*, 59 (2008) 882-901.

- [123] A.C. Dolphin, A short history of voltage-gated calcium channels, *British journal of pharmacology*, 147 Suppl 1 (2006) S56-62.
- [124] W.A. Catterall, Structure and regulation of voltage-gated Ca^{2+} channels, *Ann. Rev. Cell. Dev. Biol.*, 16 (2000) 521-555.
- [125] M.R. Tadross, R.W. Tsien, D.T. Yue, Ca^{2+} channel nanodomains boost local Ca^{2+} amplitude, *Proc Natl Acad Sci U S A*, 110 (2013) 15794-15799.
- [126] P.J. Adams, R.L. Rungta, E. Garcia, A.M. van den Maagdenberg, B.A. MacVicar, T.P. Snutch, Contribution of calcium-dependent facilitation to synaptic plasticity revealed by migraine mutations in the P/Q-type calcium channel, *Proc Natl Acad Sci U S A*, 107 (2010) 18694-18699.
- [127] K. Leal, S. Mochida, T. Scheuer, W.A. Catterall, Fine-tuning synaptic plasticity by modulation of $\text{CaV}2.1$ channels with Ca^{2+} sensor proteins, *Proc Natl Acad Sci U S A*, 109 (2012) 17069-17074.
- [128] C. Christel, A. Lee, Ca^{2+} -dependent modulation of voltage-gated Ca^{2+} channels, *Biochim Biophys Acta*, 1820 (2012) 1243-1252.
- [129] M. Ben-Johny, D.T. Yue, Calmodulin regulation (calmodulation) of voltage-gated calcium channels, *J. Gen. Physiol.*, 143 (2014) 679-692.
- [130] B.Z. Peterson, DeMaria, C.D. and Yue, D.T., Calmodulin is the Ca^{2+} sensor for Ca^{2+} -dependent inactivation of L-type calcium channels., *Neuron*, 22 (1999) 549-558.
- [131] R.D. Zuhlke, G.S. Pitt, K. Deisseroth, R.Y. Tsien, H. Reuter, Calmodulin supports both inactivation and facilitation of L-type calcium channels, *Nature*, 399 (1999) 159-162.
- [132] M.G. Erickson, B.A. Alseikhan, B.Z. Peterson, D.T. Yue, Preassociation of calmodulin with voltage-gated Ca^{2+} channels revealed by FRET in single living cells, *Neuron*, 31 (2001) 973-985.
- [133] M.G. Erickson, H. Liang, M.X. Mori, D.T. Yue, FRET two-hybrid mapping reveals function and location of L-type Ca^{2+} channel CaM preassociation, *Neuron*, 39 (2003) 97-107.
- [134] G.S. Pitt, R.D. Zuhlke, A. Hudmon, H. Schulman, H. Reuter, R.W. Tsien, Molecular basis of calmodulin tethering and Ca^{2+} -dependent inactivation of L-type Ca^{2+} channels., *J. Biol. Chem.*, 276 (2001) 30794-30802.
- [135] C.D. DeMaria, T.W. Soong, B.A. Alseikhan, R.S. Alvania, D.T. Yue, Calmodulin bifurcates the local Ca^{2+} signal that modulates P/Q-type Ca^{2+} channels, *Nature*, 411 (2001) 484-489.
- [136] A. Lee, H. Zhou, T. Scheuer, W.A. Catterall, Molecular determinants of Ca^{2+} /calmodulin-dependent regulation of $\text{Ca(v)}2.1$ channels, *Proc Natl Acad Sci U S A*, 100 (2003) 16059-16064.
- [137] H. Liang, C.D. DeMaria, M.G. Erickson, M.X. Mori, B.A. Alseikhan, D.T. Yue, Unified mechanisms of Ca^{2+} regulation across the Ca^{2+} channel family, *Neuron*, 39 (2003) 951-960.

- [138] E.Y. Kim, C.H. Rumpf, Y. Fujiwara, E.S. Cooley, F. Van Petegem, D.L. Minor, Jr., Structures of CaV2 Ca²⁺/CaM-IQ domain complexes reveal binding modes that underlie calcium-dependent inactivation and facilitation, *Structure*, 16 (2008) 1455-1467.
- [139] M.X. Mori, C.W. Vander Kooi, D.J. Leahy, D.T. Yue, Crystal structure of the CaV2 IQ domain in complex with Ca²⁺/calmodulin: high-resolution mechanistic implications for channel regulation by Ca²⁺, *Structure*, 16 (2008) 607-620.
- [140] A. Lee, R.E. Westenbroek, F. Haeseleer, K. Palczewski, T. Scheuer, W.A. Catterall, Differential modulation of Cav2.1 channels by calmodulin and Ca²⁺-binding protein 1., *Nature Neuroscience*, 5 (2002) 210-217.
- [141] S. Oz, A. Benmocha, Y. Sasson, D. Sachyani, L. Almagor, A. Lee, J.A. Hirsch, N. Dascal, Competitive and non-competitive regulation of calcium-dependent inactivation in CaV1.2 L-type Ca²⁺ channels by calmodulin and Ca²⁺-binding protein 1, *J Biol Chem*, 288 (2013) 12680-12691.
- [142] F. Findeisen, C.H. Rumpf, D.L. Minor, Jr., Apo States of Calmodulin and CaBP1 Control CaV1 Voltage-Gated Calcium Channel Function through Direct Competition for the IQ Domain, *J Mol Biol*, 425 (2013) 3217-3234.
- [143] N.J. Lautermilch, A.P. Few, T. Scheuer, W.A. Catterall, Modulation of Ca_v2.1 channels by the neuronal calcium-binding protein visinin-like protein-2., *J. Neurosci.*, 25 (2005) 7062-7070.
- [144] E. Nanou, G.Q. Martinez, T. Scheuer, W.A. Catterall, Molecular Determinants of Modulation of CaV2.1 Channels by Visinin-like Protein 2, *J Biol Chem*, 287 (2012) 504-513.
- [145] T. Tsujimoto, A. Jeromin, N. Satoh, J.C. Roder, T. Takahashi, Neuronal calcium sensor 1 and activity-dependent facilitation of P/Q-type calcium channel currents at presynaptic nerve terminals., *Science*, 295 (2002) 2276-2279.
- [146] J.L. Weiss, D.A. Archer, R.D. Burgoyne, NCS-1/frequenin functions in an autocrine pathway regulating Ca²⁺ channels in bovine adrenal chromaffin cells., *J. Biol. Chem.*, 275 (2000) 40082-40087.
- [147] J.L. Weiss, R.D. Burgoyne, Voltage-independent inhibition of P/Q-type Ca²⁺ channels in adrenal chromaffin cells via a neuronal Ca²⁺ sensor-1-dependent pathway involves Src-family tyrosine kinase., *J. Biol. Chem.*, 276 (2001) 44804-44811.
- [148] J.S. Dason, J. Romero-Pozuelo, L. Marin, B.G. Iyengar, M.K. Klose, A. Ferrus, H.L. Atwood, Frequentin/NCS-1 and the Ca²⁺-channel α -subunit co-regulate synaptic transmission and nerve-terminal growth, *J Cell Sci*, 122 (2009) 4109-4121.
- [149] L.Y. Lian, S.R. Pandalaneni, P.A. Todd, V.M. Martin, R.D. Burgoyne, L.P. Haynes, Demonstration of Neuronal Calcium Sensor-1 binding to the Cav2.1 P/Q-type calcium channel, *Biochemistry*, 53 (2014) 6052-6062.

- [150] A.L. Tippens, A. Lee, Caldendrin, a neuron-specific modulator of Cav1.2 (L-type) Ca^{2+} channels, *J Biol Chem*, 282 (2007) 8464-8473.
- [151] H. Zhou, S.-A. Kim, E.A. Kirk, A.L. Tippens, H. Sun, F. Haeseleer, A. Lee, Ca^{2+} -binding protein-1 facilitates and forms a postsynaptic complex with Cav1.2 (L-type) Ca^{2+} channels., *J. Neurosci.*, 24 (2004) 4698-4708.
- [152] P.P. Reddy, V. Raghuram, J. Hradsky, C. Spilker, A. Chakraborty, Y. Sharma, M. Mikhaylova, M.R. Kreutz, Molecular Dynamics of the Neuronal EF-Hand Ca^{2+} -Sensor Caldendrin, *PLoS ONE*, 9 (2014) e103186.
- [153] K.Y. Kim, E.S. Scholl, X. Liu, A. Shepherd, F. Haeseleer, A. Lee, Localization and expression of CaBP1/caldendrin in the mouse brain, *Neuroscience*, 268 (2014) 33-47.
- [154] F. Rieke, A. Lee, F. Haeseleer, Characterization of Ca^{2+} -binding protein 5 knockout mouse retina, *Invest Oph Vis Sci*, 49 (2008) 5126-5135.
- [155] P.S. Yang, B.A. Alseikhan, H. Hiel, L. Grant, M.X. Mori, W. Yang, P.A. Fuchs, D.T. Yue, Switching of Ca^{2+} -dependent inactivation of Ca(V)1.3 channels by calcium binding proteins of auditory hair cells, *J Neurosci*, 26 (2006) 10677-10689.
- [156] P.S. Yang, M.B. Johny, D.T. Yue, Allosteric Ca^{2+} channel modulation by calcium-binding proteins, *Nat Chem Biol*, 10 (2014) 231-238.
- [157] G. Cui, A.C. Meyer, I. Calin-Jageman, J. Neef, F. Haeseleer, T. Moser, A. Lee, Ca^{2+} -binding proteins tune Ca^{2+} -feedback to Cav1.3 channels in mouse auditory hair cells, *J Physiol*, 585 (2007) 791-803.
- [158] A. Lee, A. Jimenez, G. Cui, F. Haeseleer, Phosphorylation of the Ca^{2+} -binding protein CaBP4 by protein kinase C zeta in photoreceptors, *J Neurosci*, 27 (2007) 12743-12754.
- [159] F. Haeseleer, Y. Imanishi, T. Maeda, D.E. Possin, A. Maeda, A. Lee, F. Rieke, K. Palczewski, Essential role of Ca^{2+} -binding protein 4, a Cav1.4 channel regulator in photoreceptor synaptic function., *Nature Neurosci.*, 7 (2004) 1079-1087.
- [160] L. Shaltiel, C. Paparizos, S. Fenske, S. Hassan, C. Gruner, K. Rotzer, M. Biel, C.A. Wahl-Schott, Complex Regulation of Voltage-dependent Activation and Inactivation Properties of Retinal Voltage-gated Cav1.4 L-type Ca^{2+} Channels by Ca^{2+} -binding Protein 4 (CaBP4), *J Biol Chem*, 287 (2012) 36312-36321.
- [161] S. Mochida, A.P. Few, T. Scheuer, W.A. Catterall, Regulation of presynaptic Ca(V)2.1 channels by Ca^{2+} sensor proteins mediates short-term synaptic plasticity, *Neuron*, 57 (2008) 210-216.
- [162] W.A. Catterall, K. Leal, E. Nanou, Calcium Channels and Short-term Synaptic Plasticity, *J Biol Chem*, 288 (2013) 10742-10749.
- [163] H. Zhou, K. Yu, K.L. McCoy, A. Lee, Molecular mechanism for divergent regulation of Cav1.2 Ca^{2+} channels by calmodulin and Ca^{2+} -binding protein-1., *J Biol Chem*, 280 (2005) 29612-29619.

- [164] C. Zeitz, B. Kloeckener-Gruissem, U. Forster, S. Kohl, I. Magyar, B. Wissinger, G. Matyas, F.X. Borruat, D.F. Schorderet, E. Zrenner, F.L. Munier, W. Berger, Mutations in CABP4, the gene encoding the Ca²⁺-binding protein 4, cause autosomal recessive night blindness, *Am J Hum Genet*, 79 (2006) 657-667.
- [165] K.W. Littink, M.M. van Genderen, R.W. Collin, S. Roosing, A.P. de Brouwer, F.C. Riemsdag, H. Venselaar, A.A. Thiadens, C.B. Hoyng, K. Rohrschneider, A.I. den Hollander, F.P. Cremers, L.I. van den Born, A novel homozygous nonsense mutation in CABP4 causes congenital cone-rod synaptic disorder, *Invest Ophth Vis Sci*, 50 (2009) 2344-2350.
- [166] J.C. Hoda, F. Zaghetto, A. Koschak, J. Striessnig, Congenital stationary night blindness type 2 mutations S229P, G369D, L1068P, and W1440X alter channel gating or functional expression of Ca(v)1.4 L-type Ca²⁺ channels, *J Neurosci*, 25 (2005) 252-259.
- [167] I. Schrauwen, S. Helfmann, A. Inagaki, F. Predoehl, M.A. Tabatabaiefar, M.M. Picher, M. Sommen, C.Z. Seco, J. Oostrik, H. Kremer, A. Dheedene, C. Claes, E. Fransen, M.H. Chaleshtori, P. Coucke, A. Lee, T. Moser, G. Van Camp, A Mutation in CABP2, Expressed in Cochlear Hair Cells, Causes Autosomal-Recessive Hearing Impairment, *Am J Hum Genet*, 91 (2012) 636-645.
- [168] D.L. Minor, Jr., F. Findeisen, Progress in the structural understanding of voltage-gated calcium channel (CaV) function and modulation, *Channels (Austin)*, 4 (2010) 459-474.
- [169] F. Van Petegem, F.C. Chatelain, D.L. Minor, Jr., Insights into voltage-gated calcium channel regulation from the structure of the CaV1.2 IQ domain-Ca²⁺/calmodulin complex, *Nat Struct Mol Biol*, 12 (2005) 1108-1115.
- [170] J.L. Fallon, D.B. Halling, S.L. Hamilton, F.A. Quiocho, Structure of calmodulin bound to the hydrophobic IQ domain of the cardiac Ca(v)1.2 calcium channel, *Structure*, 13 (2005) 1881-1886.
- [171] M. Ben Johny, P.S. Yang, H. Bazzazi, D.T. Yue, Dynamic switching of calmodulin interactions underlies Ca²⁺ regulation of CaV1.3 channels, *Nat Commun*, 4 (2013) 1717.
- [172] Q. Wang, P. Zhang, L. Hoffman, S. Tripathi, D. Homouz, Y. Liu, M.N. Waxham, M.S. Cheung, Protein recognition and selection through conformational and mutually induced fit, *Proc Natl Acad Sci U S A*, 110 (2013) 20545-20550.
- [173] L.P. Haynes, G.M.H. Thomas, R.D. Burgoyne, Interaction of neuronal calcium sensor-1 and ARF1 allows bidirectional control of phosphatidylinositol 4-kinase beta and TGN-plasma membrane traffic., *J Biol. Chem.*, 280 (2005) 6047-6054.
- [174] T. Sippy, A. Cruz-Martin, A. Jeromin, F.E. Schweizer, Acute changes in short-term plasticity at synapses with elevated levels of neuronal calcium sensor-1., *Nature Neurosci.*, 6 (2003) 1031-1038.
- [175] F. Gambino, A. Pavlowsky, A. Begle, J.L. Dupont, N. Bahi, R. Courjaret, R. Gardette, H. Hadjkacem, H. Skala, B. Poulain, J. Chelly, N. Vitale, Y. Humeau, IL1-receptor accessory

protein-like 1 (IL1RAPL1), a protein involved in cognitive functions, regulates N-type Ca^{2+} -channel and neurite elongation, *Proc Natl Acad Sci U S A*, 104 (2007) 9063-9068.

[176] G. Navarro, J. Hradsky, C. Lluís, V. Casado, P.J. McCormick, M.R. Kreutz, M. Mikhaylova, NCS-1 associates with adenosine A(2A) receptors and modulates receptor function, *Front Mol Neurosci*, 5 (2012) 53.

[177] C. Schlecker, W. Boehmerle, A. Jeromin, B. Degray, A. Varshney, Y. Sharma, K. Szigeti-Buck, B.E. Ehrlich, Neuronal calcium sensor-1 enhancement of InsP(3) receptor activity is inhibited by therapeutic levels of lithium, *J Clin Invest*, 116 (2006) 1668-1674.

[178] T.Y. Nakamura, A. Jeromin, K. Mikoshiba, S. Wakabayashi, Neuronal calcium sensor-1 promotes immature heart function and hypertrophy by enhancing Ca^{2+} signals, *Circ Res*, 109 (2011) 512-523.

[179] H. Hui, D. McHugh, M. Hannan, F. Zeng, S.Z. Xu, S.U. Khan, R. Levenson, D.J. Beech, J.L. Weiss, Calcium-sensing mechanism in TRPC5 channels contributing to retardation of neurite outgrowth, *J Physiol*, 572 (2006) 165-172.

[180] R. Chattopadhyaya, W.E. Meador, A.R. Means, F.A. Quiocho, Calmodulin structure refined at 1.7 Å resolution, *J Mol Biol*, 228 (1992) 1177-1192.

Table 1 Key functionally-characterised target proteins regulated by NCS-1

Target	Ca^{2+} -bound or apo	Effect on target	Functional consequences	References
ARF1	Ca^{2+} -bound	Competes for PI4KIII β activation	Regulation of TGN to plasma membrane traffic	[117, 173]
α_1 subunit of $\text{Ca}_v2.1$	Ca^{2+} -bound	Activates Ca^{2+} -dependent facilitation of channel	Increases facilitation of neurotransmitter release	[145, 174]
Dopamine D2 receptor	Ca^{2+} -bound	Inhibits internalisation of receptor	Promotes spatial memory formation	[70, 74]
ILIRAPL1	Ca^{2+} -bound	?	Regulates N-type channels,	[84, 175]

PI4KIII β	Ca ²⁺ -bound	Activates the enzyme	secretion and neurite elongation Regulation of TGN to plasma membrane traffic	[64, 173]
Adenosine A2 receptor	Apo	?	Increases receptor signalling	[176]
GRK2	Apo	Inhibits kinase activity	Inhibits receptor internalisation	[74]
InsP ₃ receptor	Apo	Enhances receptor activity	Increases calcium signalling in neurons and heart	[177, 178]
Ric8A	Apo	?	Increases synapse number and synaptic release probability	[102]
TRPC5	Apo	Activates channel	Retards neurite growth	[179]

Table 2. Summary of the direct regulation of Ca²⁺-dependent facilitation (CDF) or Ca²⁺-dependent inhibition (CDI) of neuronal VGCCs by Ca²⁺ sensors

VGCC	Type	CDF	CDI	References
Ca _v 1.2	L-type	CaBP1	CaM, CaBP1↓ Caldendrin↓ CaBP5↓	[130, 131, 150, 151, 154]
Ca _v 1.3	L-type		CaM, CaBP1,2,3,& 4↓	[155-158, 167]
Ca _v 1.4	L-type		CaBP4↓	[160]
Ca _v 2.1	P/Q-type	CaM, VILIP-2, NCS-1, CaBP1↓	CaM, CaBP1, VILIP-2↓	[135, 136, 140, 143, 145, 146]
Ca _v 2.2	N-type		CaM	[137]
Ca _v 2.3	R-type		CaM	[137]

Figure Legends

Figure 1. Known target proteins for NCS-1 indicating interactions that require the Ca^{2+} -bound or the apo form of NCS-1. The interactions shown include ones that are based only *in vitro* binding assays as well as interactions that have been substantiated and shown to have physiological relevance in functional studies (marked with an asterisk).

Figure 2. The role of NCS-1 in the regulation of dopamine D2 receptors. Agonist occupancy of the receptors results in inhibition of adenylate cyclase (AC) through the inhibitory G-protein Gi . In addition, dopamine D2 receptors undergo desensitisation following agonist binding due to their internalization by endocytosis. This requires phosphorylation of the receptor by a G-protein coupled receptor kinase (GRK). The role of NCS-1 is to bind both the D2 receptor C-terminal tail and also GRK. Phosphorylation of the receptor is inhibited by NCS-1 and it is not internalised but instead remains at the cell surface. Inhibition of NCS-1/D2R interaction will allow receptor desensitisation.

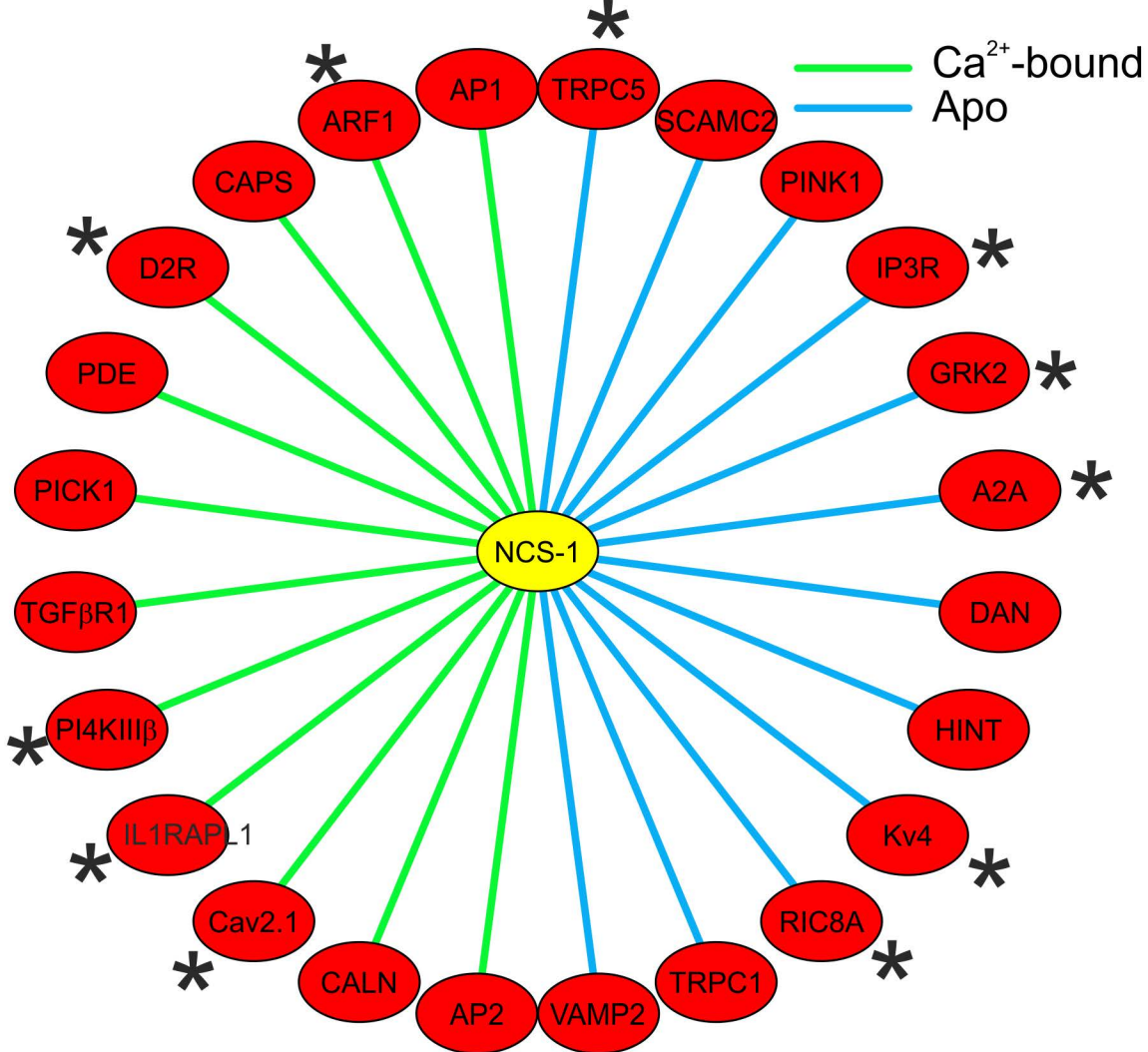
Figure 3. Key residues for target protein interactions in the hydrophobic groove of NCS proteins identified from structural studies and amino acids identified for mutagenesis in *C. elegans* NCS-1. (A) Hydrophobic residues implicated in target protein interactions are conserved in *C. elegans* NCS-1. Those residues that directly make contact with target proteins in structurally characterised complexes are indicated above the sequence of *C. elegans* NCS-1. Residues selected for mutagenesis are boxed. (B) Position of residues selected for mutagenesis in the predicted NCS-1 structure. The selected amino acids are within the hydrophobic groove are shown in red in a surface representation of a model structure for *C. elegans* NCS-1 based on the crystal structure of human NCS-1 (PDB1G8I). Adapted from Martin et al 2013 [60].

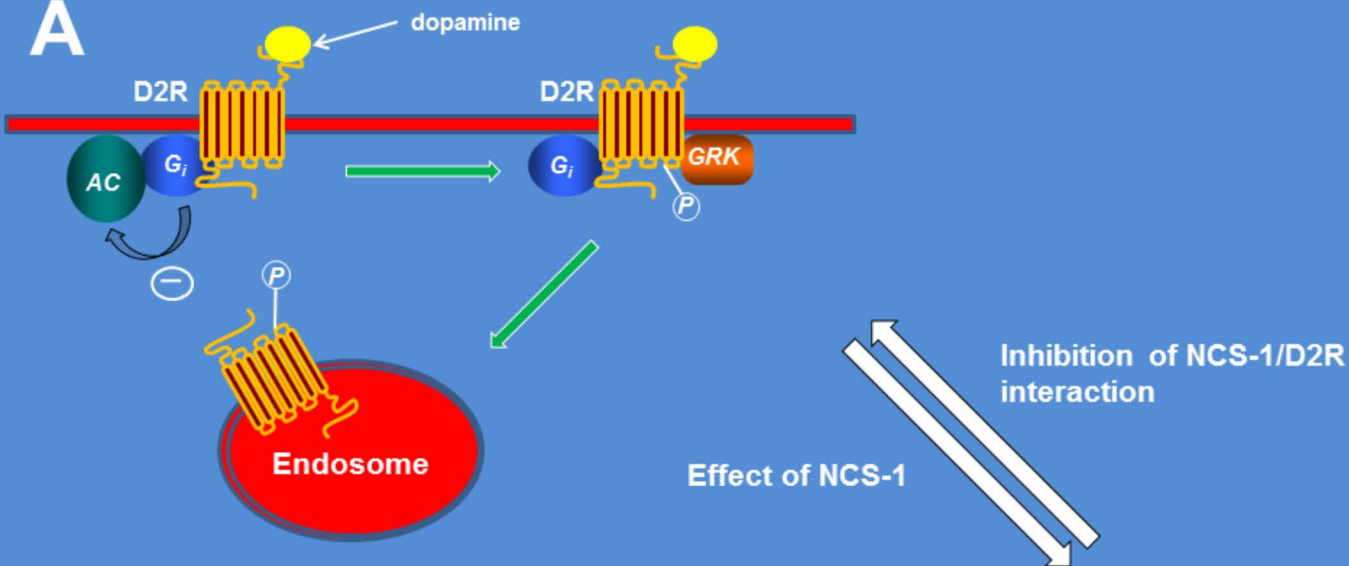
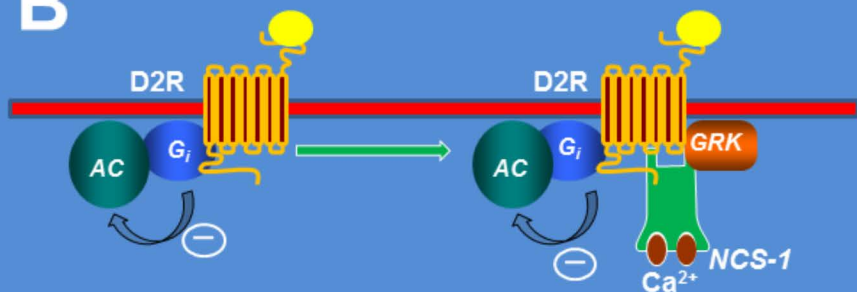
Figure. 4 Domain structure of the CaBP family of Ca^{2+} sensors compared to calmodulin. Unlike calmodulin each of the CaBPs has at least one inactive EF-hand. Some are membrane targeted due to N-terminal myristoylation whereas CaBP7 and CaBP8 possess C-terminal transmembrane domains that determine their membrane association [114, 115]

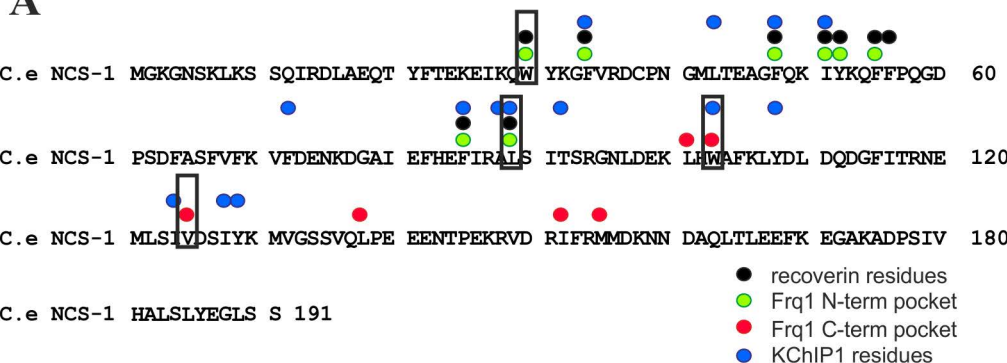
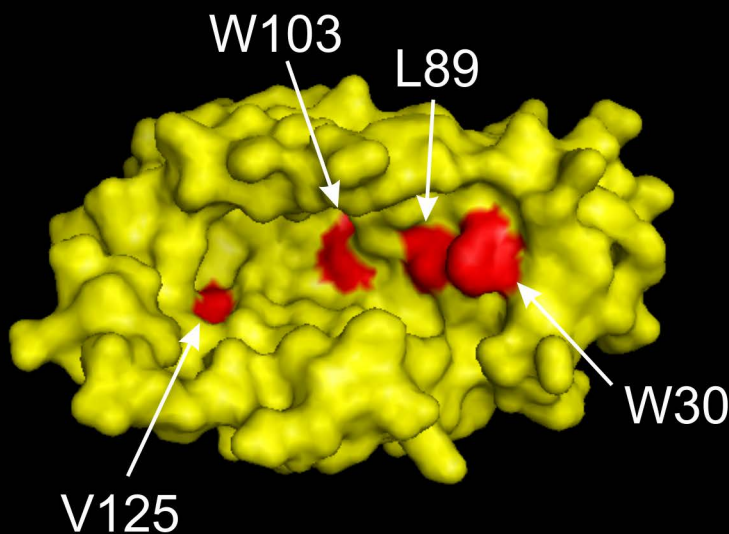
Figure. 5. Comparison of the N-terminal domain of CaBP7 with the C-terminal domains of CaBP1 and calmodulin. Ribbon representation of Ca²⁺-bound CaBP7 NTD (PDB code: 2LV7, residues 30-100, purple) superposed with (A) Ca²⁺-bound CaBP1 CTD (cyan, PDB code: 2LAP) and (B) Ca²⁺-bound CaM CTD (yellow, PDB code 1CLL, residues 80-147). Red spheres represent bound Ca²⁺. Space-filling representations of (C) Ca²⁺-bound CaBP7 N-terminal domain (PDB code: 2LV7, residues 30-100), (D) Ca²⁺-bound CaBP1 C-terminal domain (PDB code: 2LAP) and (E) Ca²⁺-bound CaM C-terminal domain (PDB code: 1CLL, residues 80-147) showing the predicted ligand binding face. Acidic residues and basic residues are shown in red and blue, respectively. Hydrophobic residues are shown in yellow with the exception of Met residues which are highlighted in orange. Adapted from McCue et al 2012 [119].

Figure. 6. Potential binding sites on VGCCs for Ca²⁺ sensors that have been identified in the N- or C-terminal cytoplasmic domains of the α_1 subunits of various types of VGCCs. These include a site in the N-terminus and the IQ and calmodulin-binding (CBD) domains in the C-terminus. Other regions of the C-terminal domain may also be required for Ca²⁺ sensor interactions.

Figure 7. Comparison of the structures of Ca²⁺-loaded calmodulin with and without bound target peptide. The structures shown are of Ca²⁺-bound calmodulin alone ([180]) on the left or in a complex with the IQ-like domain of the Ca_v1.2 Ca²⁺-channel α_1 -subunit (PDB 2F3Z) [170] on the right with the IQ-like peptide shown in yellow.



A**B**

A**B**



Myristoylation site



Active EF-hand



Inactive EF-hand

EF1

EF2

EF3

EF4

Calmodulin

Caldendrin

CaBP1l

CaBP1s

CaBP2l

CaBP2s

CaBP4

CaBP5

CaBP7

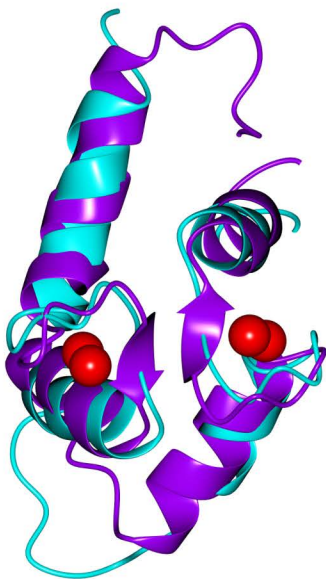
CaBP8

TMD

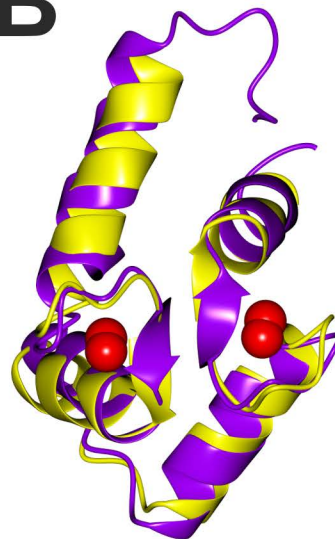
TMD

***N-terminal
variable region***

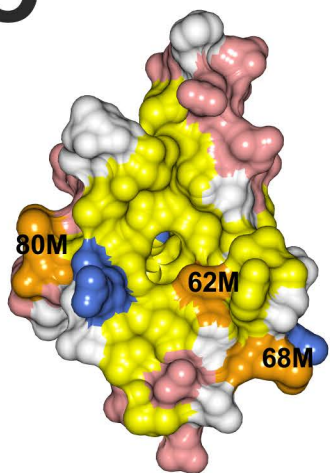
Conserved EF-domain

A

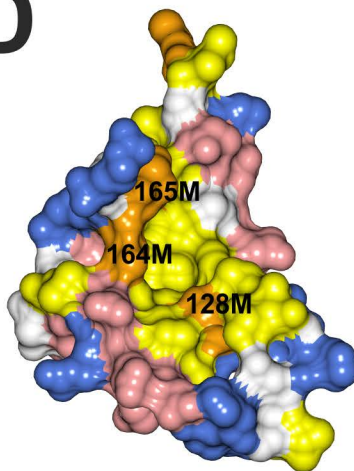
CaBP7 NTD/CaBP1 CTD

B

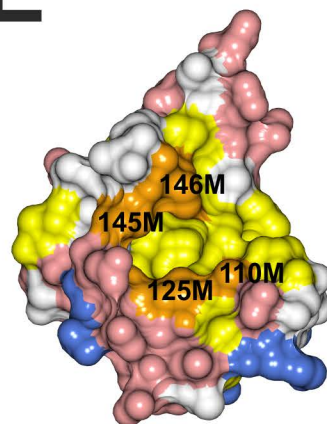
CaBP7 NTD/CaM CTD

C

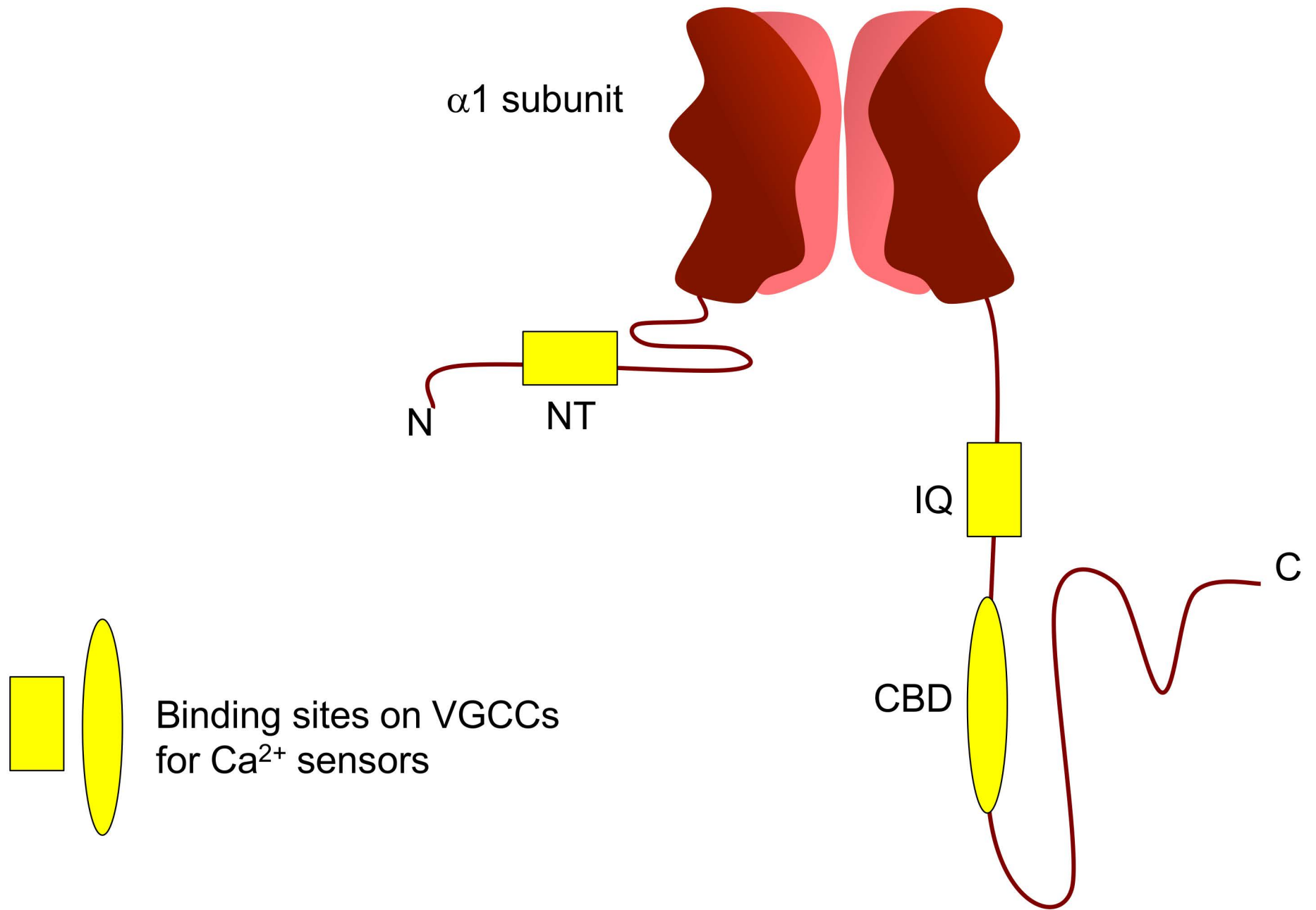
CaBP7 NTD

D

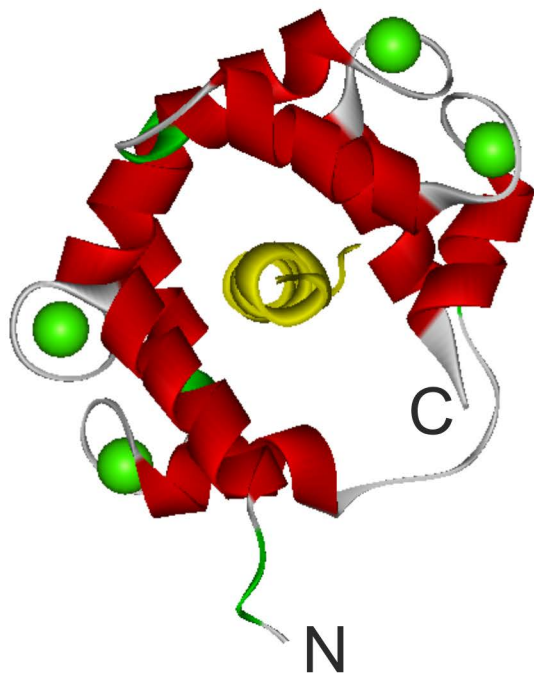
CaBP1 CTD

E

CaM CTD



N-terminal lobe



C-terminal lobe